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A RATIONAL EXAMINATION OF STREAM POLLUTION ABATEMENT

By RICHARD D. HOAK

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STREAM pollution, from all sources combined, is an extremely complex problem which has expanded gradually, largely through non-recognition of its effects or indifference to them. If it is borne in mind that the amount of pollutive substances discharged to streams has increased almost imperceptibly, in general, over many years, it is not difficult to understand how such pollution has become widespread before there has been a realization of its significance. As pollution abatement involves many conflicting factors it is desirable, from time to time, to re-examine the subject and consider its fundamentals. Numerous technical reports, treatises and results of surveys are in existence, couched in the language of the biologist, the chemist and the engineer. In this article an attempt will be made to discuss some pertinent considerations in the familiar words of the layman.

STREAM FUNCTION

The geological plan of the earth offered streams to carry away the waste products of natural processes. In conducting excess water from the land to the oceans, streams transport such a quantity of mineral and organic wastes that many watercourses support but little aquatic life and their waters are unsuitable for domestic or manufacturing uses without extensive treatment. Streams vary widely from basin to basin in their normal burden of silt, dissolved substances, organic matter and microorganisms, and the restoration of streams to their natural state by the elimina-

tion of man-made waste products would, in some areas, result in little practical improvement.

As the country was settled, towns and industries grew on the banks of rivers, for here were highways on which merchants, farmers and artisans alike depended for the transfer of products. The convenient streams also served to carry away many of the wastes of communities. The development of public sewerage systems began in the United States in 1855, and by 1860 17 per cent. of the urban population was served by sewers. This proportion increased to 35 per cent. in 1900, and by 1942 about 87 per cent. of the population living in communities of more than 100 persons was connected to sewers. This development was accompanied by an increase in the volume of industrial wastes discharged to streams. At first the dilution of the wastes was usually so large that nuisances rarely occurred; but as the population became larger and the volume of wastes increased, there gradually developed a conflict over the relative importance of different stream functions.

The important functions of streams have been classified in one large basin as (1) public health, (2) drainage, (3) navigation, (4) industry and (5) recreation. It will generally be agreed that the highest use of a stream is for domestic water supply, but the importance of other uses will depend upon the area in which the stream lies and the relative value of one use over another may change from section to section of the stream. In fact, differences of opinion as to superior use will occur along relatively short stream lengths because each stream user will have a tendency to think that his benefit is the most important. Priority of stream utility can be established only by an impartial engineering, economic and societal survey which will give proper weight to all the factors involved.

Any survey which seeks to establish a basis for the abatement of stream pollution in a given area must take cognizance of existing legal decisions. The courts generally have based their rulings upon an interpretation of the principle of reasonableness, *i.e.*, a riparian proprietor may pollute a stream to a reasonable extent. It has been held that there is no public policy in favor of industrial development which will justify, for the sake of a factory or mill, destruction of the rights of other riparian owners by pollution of a stream. But it has also been decided that a long-continued uniform custom of mill owners and manufacturers along a particular stream may make it a reasonable use for them to discharge their waste and refuse into the stream. In a stream pollution suit a defendant can not escape liability by showing that his business is of great benefit to the public or that the contaminating matter can not be disposed of without great expense; but the courts recognize the importance of business enterprises and they do not overlook the needs of important manufacturing interests nor hamper them for trifling causes. Substantial injury to lower riparian property, however, will not be permitted for the purpose of enabling a new and great industry to flourish. The tendency of the courts to rule upon the reasonableness of a discharge must be kept in mind when waste-treatment plants are planned, because what may be considered reasonable in one jurisdiction may not be so regarded in another.

Natural Purification in Streams

A stream should be looked upon as a natural biological and chemical treatment plant which operates efficiently as long as its capacity is not exceeded. Full advantage of the ability of a stream to purify itself should be taken in evaluating the possible effect of pollution. Many individuals view the discharge of any pollutant whatever as harmful, but moderate organic pollution frequently improves stream ecology (the mutual relations between organisms and their environment) by providing a food supply for the development of aquatic life which would otherwise be absent. On the other hand, there are persons who believe that the capacity of a stream for self-purification is relatively unlimited. These misconceptions have often led to dispute. When a stream is subjected to excessive pollution for a brief period, a much longer time will be required to restore its normal biochemical balance.

Two constituents of natural waters most important in self-purification of streams are alkalinity and dissolved oxygen. The former is introduced mainly by the dissolution of rocks as the water percolates through or flows over the earth, the latter is obtained from the air and from aquatic plants in the stream bed. There are many other natural agencies which affect stream economy, but all are more or less dependent on these two.

Most streams contain natural alkalinity which consists principally of the bicarbonates of lime and magnesia. Stream alkalinity varies widely over the country, from the soft waters of the middle south to the very hard waters of the Midwest. Alkalinity is important because it reacts with acidic contaminants, *e.g.*, acid mine water, and helps to maintain the stream in proper condition to support the growth of the variety of plant and animal life necessary to stream balance. Where the alkalinity of a stream is destroyed by pollutants many beneficial flora and fauna die quickly if the water remains more than slightly acid.

The water in a clean stream is generally saturated with oxygen. Organic wastes, such as domestic sewage, are chemically unstable and have a strong ten-

dency to decompose into simpler substances. If an adequate supply of oxygen is present, the decomposition occurs without creating a nuisance, and, if the waste is discharged to a stream, the stream water must supply the required oxygen. To preserve the normal functions of a stream, however, the organic load must not be allowed to increase beyond a point which would reduce the dissolved oxygen below about 50 per cent. of saturation.

Bacteria which thrive in the presence of air play a prominent role in stream purification, because they feed on unstable organic material, reducing it finally to gases, water and other compounds. The bacterial population of a stream varies directly with the food supply and where the concentration of organic substance increases in the water the bacteria multiply very rapidly. But the bacteria which reduce organic matter inoffensively also require oxygen to live, and as the waste increases and the oxygen reserve is depleted, these organisms gradually die. Finally, where the oxygen demand of the wastes continuously exceeds the oxygen available in the stream, other species of bacteria appear to which oxygen is a poison, and "anaerobic" decomposition begins. Under conditions of putrefaction a stream becomes septic and devoid of all higher forms of life; to restore a stream from this state to normal biological balance requires a long time.

Present Extent of Water Pollution

Somewhat more than half of the country's population is served by sewers, but of the sewage so collected only about 60 per cent. is treated before its discharge into streams, rivers and lakes. The sewered communities produce nearly 6,000 million gallons of waste daily. This large volume of waste corresponds to less than one half of 1 per cent. of the average daily stream flow in the United States; and if it could be equitably proportioned among these streams, it would probably be assimilated without difficulty, but, originating in selected communities, it may be an excessive local burden.

The flow of domestic sewage follows a well-defined pattern and, although its composition will vary somewhat in different localities, depending on mass habits, its contribution to the whole pollution problem can be accurately assessed and its effect can be confidently projected a reasonable distance into the future. The intensity of the pollution arising from the discharge of industrial wastes can not be evaluated in simple terms for the country at large, because such wastes vary widely in quantity and kind not only from industry to industry but from day to day in the same industry. Industrial wastes may consist of organic, inorganic, toxic, acid, alkaline or inert substances or of a combination of several of them.

Although there is no simple standard by which the intensities of industrial wastes can be compared with each other or with domestic sewage, the term "population equivalent" is ordinarily used as the most satisfactory index available. A large number of measurements has shown that the average daily volume of waste produced by one person requires 0.168 pound of oxygen for its stabilization. A determination of the oxygen demand of an industrial waste then yields a measure of its intensity in terms of an equivalent number of persons. Because organic wastes generally cause the most serious stream damage, this index is very useful for appraising the effects of such wastes and for establishing the kind and degree of treatment necessary.

The Ohio River Pollution Survey, recently completed by the U. S. Public Health Service, is the most extensive study of pollution and its effects ever undertaken for a large area in this country. The Ohio River drains 204,000 square miles, an area which has a population of more than 19 million. In this highly industrialized region about eight and one half million persons are served by sewers, but of the sewage so collected only 34 per cent. is treated before discharge. The industries in the basin produce wastes equivalent to a population of almost 10 million, and about 12 per cent. of this waste is treated along with domestic sewage at municipal plants. Measures have been taken to reduce the industrial waste originating in 808 or 50 per cent. of the 1,604 plants in the basin (exclusive of Cincinnati, where individual plant surveys were not made) which do not discharge wastes to municipal treatment works. In other words, some effort is made to reduce pollution intensity at more than 62 per cent. of the industrial plants in the Ohio River basin.

The continuous discharge of acid water from active and abandoned coal mines is an industrial waste problem of great importance. It has been estimated that the daily discharge of acid mine water in the Ohio River valley alone carries free and combined sulfuric acid equivalent[1] to 4,900 tons of 100 per cent. acid, of which about 50 per cent. flows from abandoned mines and most of the remainder from worked-out sections of active mines. The Federal coal-mine sealing project (1935–1938) brought about a reduction of approximately 25 per cent. of the acid originally present in mine water, but it is reported that lack of a maintenance force has resulted in many of the seals becoming ineffective. Waste pickle liquor, the spent acid which is produced in removing scale from steel prior to further processing, is an industrial waste to which objections have been made from time to time. In the Ohio River basin, which contains a heavy concentration of steel mills, the average daily

discharge of free acid from this source amounts to about 168 tons. Decomposition of the iron salts dissolved in this liquor slowly produces more free acid upon discharge to streams, but the total acid so introduced is small compared with that from acid mine drainage.

Acid mine water occupies the anomalous position of being an inorganic pollutant and, at the same time, of reducing the immediate effects of organic pollution. Acid water exerts a germicidal effect which inhibits the decomposition of organic matter, and the salts of iron and aluminum it contains coagulate and precipitate organic substances in watercourses. The situation at Pittsburgh, where all the sewage of the community is discharged untreated into the Allegheny and Monongahela Rivers, exemplifies this condition. Here, were it not for the presence of acid mine water and other acidic wastes in the rivers, sewage pollution would create an offensive odor nuisance at Pittsburgh and would seriously overburden downstream water-purification plants. That this situation is against the public interest, however, is evidenced by the fact that during drought periods intestinal disorders have developed in communities below Pittsburgh which depend on the Ohio River as a source of public water supply. Reduction in the intensity of mine acids by flow augmentation reservoirs coupled with effective mine sealing, and treatment of the sewage discharged by Pittsburgh, would be a long step toward the restoration of the Ohio to a healthy condition.

The volume of acid mine water is steadily increasing, and, unless or until the concentration of acid in streams can be appreciably reduced there appears to be no reasonable justification for requiring the elimination of minor sources of stream pollution. Operators of water purification plants, however, can not depend upon the bacteriostatic effect of acid water because during a freshet the acidity may be neutralized and its germicidal action eliminated. A rapid increase in the rate of river flow also flushes out accumulated sludge banks, thereby imposing a still heavier burden on water-treatment facilities.

The Problem of Waste Treatment

Waste treatment is a problem alike to municipality and industry. It is expensive and usually yields no by-products of substantial value. Delay in the installation of waste treatment is notable in the case of communities, where the taxpayer appears to receive no immediate benefit from sewage treatment. Federal financial aid acts as a stimulus to municipalities faced with problems in stream sanitation, as demonstrated by the fact that from 1932 to 1938 more progress was made in municipal sanitation than in the previous 25 years.

The treatment of domestic sewage is so well standardized by practice that plants can be installed, if the expense can be borne, for any degree of purification demanded by circumstances. Industrial waste treatment is a particularly difficult problem because standardized processes do not exist for more than a few wastes. The characteristics, volume and concentration of industrial wastes may change, even in the same plant, from day to day, and the variety of wastes is so great that almost every appropriate method of disposal constitutes an individual problem.

Research has been and is now in progress in many industries with the object of developing satisfactory waste-treatment processes. Typical of this kind of research is the current program of the American Iron and Steel Institute, established at Mellon Institute in 1938 for the investigation and development of feasible procedures for eliminating that part of stream pollution attributed to the discharge of steel mill wastes. The paper and pulp industry has initiated a comprehensive research program to develop new methods for processing its wastes and to coordinate the efforts of individual companies in dealing with their particular disposal problems. The leather tanning industry has made a survey of methods being employed to treat its wastes and is promoting the study of improved treatment processes. The food and beverage industries, probably the largest producers of organic wastes, are constantly alert to recognize and adapt new procedures for the disposal of their wastes, which are among the most difficult to treat successfully. In similar fashion other industries plan investigations in disposing of their waste waters. Only infrequently does industrial waste treatment become a profitable enterprise, although in some cases by-products can be recovered which will bear a portion of the cost of processing. Other things being equal, it is obvious that a manufacturer who is required to expend considerable sums for the installation and operation of a treatment plant is at a disadvantage over one who escapes this responsibility.

For some industrial wastes no completely satisfactory treatment process is yet known; others can be handled only at considerable expense. The cost of waste treatment has an important effect on industrial economy, particularly in highly competitive fields, and the operation of acceptable works might result in some manufacturers actually producing goods at a loss. It is clear that where a substantial segment of a community depends upon an industry operating on a small profit margin and that industry is required to install a costly waste-treatment plant the domestic economy of many workers may be seriously affected. This does not mean that the discharge of the pollutants should be universally tolerated, but it

does suggest that every major pollution-abatement program be examined scientifically in the light of the net effect on the welfare of the community as a whole. Admittedly it is frequently difficult to balance the value of such an intangible as recreation against family income, but generally a competent and complete survey will enable a just decision to be reached.

The expansion of industry to fill quickly the colossal demands of war has resulted in an unavoidable increase in the discharge of waste waters to streams, just as it has added to air contamination by smoke. This temporary augmentation of pollution by industrial waste has served to emphasize the desirability of treatment as a means of reducing the effects of all forms of contamination.

Public Attitude toward Treatment

The unquestionably plain importance of prosecuting the war with all speed and with all available resources had made it practically impossible to consider the erection of new, or the extension of existing, treatment works. Allocation of materials of construction has been denied except in those apparently rare situations where the wastes could be shown to have a direct effect on public health. Further, the engineering staffs of state departments of health have been much reduced by the requirements of the Armed Forces for qualified sanitary engineers. The consequence of this depletion of public health engineers has been a relaxation in the supervision of existing works and a diminution of opportunities for industries and municipalities to receive advice on their problems.

State sanitary engineers have indicated their intention of adopting an aggressive policy toward industrial waste pollution of surface waters as soon as equipment and personnel again become available for the construction and operation of waste-treatment works. The demands made upon industry by state departments of health are normally reasonable because such agencies weigh the advantages to be obtained against the feasibility and cost of treatment and its economic effect on the industry involved. Primarily, they are influenced by the principle of the greatest good to the greatest number.

In the movement to purify the streams of the country, the desires and wishes of the sportsmen are quite positive. Few persons are unsympathetic with the general objectives of that group, within the limits of feasibility. But it must be remembered that the discharge of wastes is often not the only cause for the disappearance of aquatic life from our inland waters. Due attention must be given to other factors. For example, the temperature of stream water is raised by deforestation and the increase may be great enough to create an unfavorable environment for some species of fish. Clearing hillsides of trees and undergrowth increases the rate at which rainfall runs off the land and this condition results in scouring stream bottoms and the deposition of sand bars, thereby destroying the feeding and breeding grounds of fish. Aquatic fauna are modified as a result of soil erosion which changes the chemical and physical characteristics of streams. Conditions are adversely affected by canalization of rivers, construction of navigation and flood control dams, straightening and rip-rapping of river banks and curtailment of marshy, lateral areas along streams by drainage and channel improvements. Finally, it should not be forgotten that overfishing, even in uncontaminated waters, has been an important factor in the depletion of our aquatic resources.

The average person believes, in a broad way, that pollution abatement is desirable provided it is accomplished at no immediate cost to him. Accounts of pollution damage, particularly fish-killings, are much more newsworthy than stories of pollution eliminated, and the public has little appreciation of the complexity or the cost of a general pollution abatement program. It has been estimated that two and one quarter billion dollars worth of treatment plants, about half municipal and half industrial, would have to be built to correct the major but not all sources of pollution in the United States. If these plants were built, there would be an annual operating and financing cost of about 116 million dollars over a thirty-year period. A program even of this magnitude would not result in the complete elimination of pollution, but, applied wholly or in part, it might merit consideration in evaluating postwar unemployment.

Administration of Pollution Abatement

It is inevitable that a subject as broad as stream pollution should have its political aspects. Various authorities are interested, and pertinent rules and regulations have been promulgated by state and federal agencies. Legislation has been on the scene for a long time, more legislation is being advocated annually. Under certain conditions, loans or grants-in-aid have been available to municipalities faced with serious problems in sewage treatment. While it is not the purpose of this article to deal comprehensively with the many-sided political and jurisdictional questions involved, there is one observation, based upon history, which seems fitting at this time, namely, that legislation alone can hardly be wise enough to provide for an adequate, economically feasible and rational remedy and at the same time preserve the principle of the greatest good to the greatest number of people. Research, education and finance are at least

as important as legislation in the abatement of stream pollution. Research and education must point the way, and finance furnish the wherewithal. Without them, attempts to apply mandatory corrective measures are apt to be abortive. Once the many answers as to proper methods of treatment are known and appropriate standards are set to govern the condition of streams in various localities, it may make little difference from whence the authority of administration flows, provided the administration is practicable and equitable.

The Future of Pollution Abatement

There is no gainsaying the fact that the discharge of waste waters into streams imposes a burden upon them which, under certain circumstances, may be undesirable. To hope, however, for the restoration of all streams to a state of pristine purity in thickly settled and highly industrialized sections of the country is probably beyond the realm of practical attainment. In some districts there are sources of contamination, resulting in part from past activities, which have been long established and may not be susceptible to engineering and chemical treatment. They will be factors even though wastes currently produced are rendered harmless.

It has been proposed by some students of the subject that streams be divided into several classes according to superior overall function. Into the first group would fall those streams which have been preserved in nearly their natural condition and to serve as sources of public water supply or for recreation. The purity of such streams is already so jealously guarded by various associations and by state health departments that they are unlikely ever to become contaminated. The second group would include those streams which serve for the disposal of sewage and industrial waste after treatment and for public water supply after purification. This class would comprise most of the streams in industrial areas except those carrying a heavy burden of acid mine water. Long reaches of such streams would provide recreational areas and wildlife refuges because waste treatment would be adequate to preserve a natural stream balance. Many watercourses in this group will have the benefit of additional waste treatment facilities, which are being installed as acceptable treatment processes are developed and money to finance them becomes available. A third group would consist of those streams now heavily polluted and unsuited for public water supply into which wastes might be discharged after treatment adequate to prevent nuisances. Actual stream mileage in this class is relatively small.

The network of streams with which industrial districts are endowed played an important role in our national development and can not be considered apart from their collective function in the overall economy. Over a period of years, contamination increased. Over a period of years, with the finding of feasible methods for the treatment of wastes, contamination should decrease. Chemistry and engineering, through research, have evolved many valuable by-products from former wastes. They have marked a course for industry to follow. With the continuation of research work supported by many branches of industry, there is the hope that reasonably economical processes may be developed whereby industrial wastes may be handled satisfactorily.

OBITUARY

THOMAS FRANKLIN HOLGATE

Dean Thomas F. Holgate, who had long held a distinguished place in American science and education, died on April 10 at the age of eighty-six years. He had been associated with Northwestern University for fifty-two years—as professor of mathematics, as dean of the college, as acting president of the university and as professor and dean emeritus. After retirement from teaching at the age of seventy-five, he continued to live in his home near the campus, maintaining his scholarly interests and participating in religious and civic affairs to the end of his long and fruitful life.

Born in Hastings County, Ontario, on April 8, 1859, he received a bachelor's degree from the University of Toronto in 1884, and a master's degree in 1889. He entered Clark University for advanced study in mathematics in 1890, receiving his doctorate three years later. He accepted a position as instructor in mathematics at Northwestern University in the summer of 1893, and was promoted to a professorship in applied mathematics the following year.

When he came to the Midwest in 1893, the Chicago World's Fair was in progress, and the new University of Chicago had recently been opened. Working with an enthusiastic and brilliant group of young mathematicians—including such men as White, Moore, Bolza, Maschke, Miller and Van Vleck—he took part in major scientific developments. He attended the first International Congress of Mathematicians (held in Chicago in connection with the Fair) and a famous series of Colloquium Lectures by Felix Klein (held in Evanston following the Congress). He was active in the formation of the Chicago Section of the Ameri-

n Mathematical Society in 1897, and he served as secretary from 1897 to 1905.

Dean Holgate published a number of mathematical pers between 1892 and 1908, his most important es dealing with certain ruled surfaces of the fourth ler. His translation of "Reye's Geometrie der ge," published in 1898, was an important contri-tion to our mathematical literature. Three years er he brought out a text-book, "Elementary Geome-y, Plane and Solid," which was widely used in high hools and colleges. In 1911 he contributed an cyclopedic monograph on "Modern Pure Geometry" J. W. A. Young's well-known volume entitled Monographs on Modern Mathematics." Because of avy administrative duties he made no further thematical contributions for many years, but in 30, at the age of seventy-one, he published his Projective Pure Geometry," an excellently written ok designed for college seniors and graduate stu-nts.

In recognition of his scientific standing, Dean Hol-te's name was starred in the first edition of "Ameri-n Men of Science," in which he was ranked among e forty leading American mathematicians in 1903. e served as a secretary of the International Congress Mathematicians at Rome in 1908.

Starting in 1902, he served for seventeen years as an of the College of Liberal Arts. To this office gave unsparingly of energy and thought. He had flair for such work, and demonstrated a remarkable bility for mastery of the details of the activities of e college and the university. Faculty and students ke found in the dean's office a genial clearing-ouse. No problem was too trivial to receive careful nsideration; none too large to be faced courage-usly. Twice he was called upon to serve as acting resident of the university, from 1904 to 1906 and gain from 1916 to 1919. It was thus his responsi-lity to lead the university during the strenuous days f World War I, and he carried out the assignment ith energy, patience and sound judgment. During ese decades of administrative service he was a aluable member of many educational and civic com-ittees, commissions and organizations. To mention nly one, he was a leader in the formation of the orth Central Association of Colleges and Secondary chools, and was its president in 1917–1918. He on a high place for himself in the annals of his niversity by his success as an administrator.

In 1919, at the age of sixty, he retired from major dministrative duties with the title of dean emeritus, ut he continued his teaching for fifteen years. He pent one year, 1921–1922, as visiting professor of athematics at the University of Nanking. This xperience served to intensify his interest in foreign tudents, by whom he was regarded as an illustrious friend and an ever available adviser. During his long teaching career he inspired a host of students by his quiet enthusiasm, his clarity of expression and his scientific integrity.

To refer to Dean Holgate's university activities alone would give an inadequate account of his life, for he was long prominent in civic and religious affairs, particularly in administrative problems of the Methodist Episcopal Church. Serving five times as a member of the General Conference of the church, while momentous changes were being considered, he became a respected and influential participant in their sessions. He also served the church as a member of its Board of Education for Negroes, giving advice and guidance to several Negro colleges in their formative stages, in recognition of which they have named buildings in his honor. From 1924 until his death he was a member of the Board of Education of the church, being treasurer of the organization for four years. From 1923 to 1925 he was president of the Chicago Church Federation. This incomplete account of the official positions which he held is indicative of the wide influence which he exerted in fields outside the sphere of the university.

Dean Holgate was thrice honored with the LL.D. degree—by the University of Illinois in 1905, by Queen's University in 1919, and by Northwestern University in 1937.

We have mentioned the high points of Dean Holgate's scientific and public career, but those who knew him well will longest remember him as a generous and considerate friend, a man to whom his friends naturally turned for assistance or sane advice. To thousands of students of Northwestern University he will forever be "the Dean," a friend who always remembered their names and continued to have a personal interest in them. All who knew him will remember him for his staunch dependability, for his unfailing poise and dignity and for his granite integrity.

Dean Holgate is survived by four children—Eleanor (Mrs. Owen Lattimore), Robert Burdette, Barbara and Frances Burdette.

E. J. MOULTON

RECENT DEATHS

THE death by suicide is reported of Dr. George Vaillant, director of the University of Pennsylvania Museum, previously of the American Museum of Natural History. He was forty-four years old.

DR. HAROLD ORVILLE WHITNALL, head of the department of geology of Colgate University, died on May 18 at the age of sixty-seven years.

DR. ELLEN B. FOOT, assistant professor of anesthesiology at the Cornell University College of Medicine

and chief of the department of anesthetics at the New York Hospital, died on May 11 at the age of thirty-two years.

DR. STANLEY WELLS KEMP, since 1936 director of the Plymouth Laboratories, England, secretary of the Marine Biological Association of the United Kingdo died on May 16. He was sixty-four years old.

DR. HAROLD HIBBERT, professor of industrial a cellulose chemistry at McGill University, who retir in 1943, died on May 4 at the age of sixty-seven yea

SCIENTIFIC EVENTS

ACTIVITY OF PARICUTIN VOLCANO

DURING March, the effusive activity of Paricutin diminished while the explosive activity increased in intensity. All the lava vents close to the western base of the cone that were active during February died early in March. Of the two principal ones, that which opened on January 12 was extinguished on March 5, by which time a narrow, branching stream had extended northward for almost a mile. The other main vent in that vicinity, which opened on February 4, continued to discharge lava until approximately March 10. It sent a longer stream northward that first cascaded down steep slopes and then buried most of Paricutin Village not already inundated by the flows of 1943 and 1944. The "Aguan vent," which opened at the south base of the cone on or about November 10, 1944, continued to emit lava throughout March, though in diminishing amount. At the vent itself no fresh lava could be seen, only an active hornito shaped like a beehive. Apparently the lava, after flowing for half a mile through a concealed tunnel close to the southeast foot of the cone, broke through its crust and set out many new tongues that spread over the earlier already solidified Aguan lava to the east of the cone. One of these new tongues was injected beneath and thus upheaved the heavily ash-laden lava erupted from the parasitic cone of Zapicho in 1943. Such sill-like injections of new into old lavas have been a recurrent phenomenon at Paricutin.

Meanwhile the explosive activity of the main cone has been unusually intense. Indeed, during the latter part of March the pall of ash was so heavy and the mantle of newly fallen material was so stirred by winds that for most of the daylight hours the cone was completely hidden from view. At night, when the winds subsided, the flanks of the cone were brilliantly lit by showers of glowing bombs.

No new lava vent has opened at Paricutin since February 4; whether or not this crescendo of explosive activity heralds a new effusion remains to be seen. When the cone was last climbed, on March 5, it contained a single, deep, runnel-shaped crater within which were two small, more or less cylindrical pipes, one of which was lined with incandescent magma.

COMMITTEE OF THE NATIONAL RESEARCH COUNCIL ON THE STUDY OF THE PARICUTIN VOLCANO

THE BAUSCH AND LOMB OPTICAL LIBRARY OF THE UNIVERSITY OF VIRGINIA

MEMBERS of the optical manufacturing indust have collaborated in recent months to bring up to da the Adolph Lomb Optical Library at the University Virginia. When this collection on the science optics was placed at the university, no provision w made for purchase of works published currently that field of learning. Consequently the passage twelve years found the library deficient in certain i portant aspects in which research has made advance When this situation was brought to the attention the optical industry, the following firms joined establishing a fund of $1,100 with which to purcha the most significant recent publications for additi to the library: the Anchor Optical Corporation, Ne York City; Applied Optical Industries, New Yo City; Bonschur and Holmes Optical Company, Phil delphia; Libbey-Owens-Ford Glass Company, Toledo the May Oil Burner Corporation, Baltimore; Minn apolis-Honeywell Regulator Company; Zenith Optic Company, Huntington; and Richard B. Tucker, '0 vice-president, Pittsburgh Plate Glass Company, Pitt burgh. Now that provision has been made for brin ing the collection up to date, a movement is under wa in the industry to establish a modest fiduciary fun the income of which will be used to purchase boo and thus ensure the continuance of the library as t outstanding collection in its field of science. Co panies or individuals wishing to have a part in th movement are advised to communicate with Dr. Har Clemons, librarian, University of Virginia, Charlotte ville, Va.

A catalogue of the library is now in process of pub lication for complimentary distribution among th members of the industry who have participated i this service. The catalogue will carry an introductio by Dr. James P. C. Southall, for many years profess of physics at Columbia University.

PITTSBURGH GEOLOGICAL SOCIETY

A REGIONAL society "to advance and disseminat geologic knowledge, and to provide a forum for geo logical problems" has been organized at Pittsburgh under the name of the Pittsburgh Geological Society. The first meeting was held on October 27, 1944. De tails of organization have been worked out at the fir

successive monthly meetings, culminating at the April meeting when the constitution and by-laws were adopted.

The organizing committee was headed by George C. Grow, Jr., who has also been selected as president of the society for the year 1945–1946. Other first-year officers are Raymond E. Birch, *vice-president;* W. B. Robinson, *secretary;* and Dr. Shailer S. Philbrick, *treasurer.* These officers, together with the following, comprise the council: Robert E. Bayles, Hugh R. Brankstone, C. H. Feldmiller, Dr. Richard M. Foose, John T. Galey, David K. Kirk and Dr. J. H. C. Martens.

Speakers at the first six meetings and their subjects follow: Major Raymond C. Moore, State Geologist of Kansas, now on leave in the Army, "Geology and Geomachy"; Dr. Kirk Bryan, Harvard University, "The Geological Antiquity of Man in America"; Dr. Carey Croneis, president, Beloit College, "Science and the Future"; Dr. Philip S. Smith, chief Alaskan geologist of the U. S. Geological Survey, "Alaska's Potential Oil Resources"; Dr. W. C. Krumbein, senior geologist, Beach Erosion Board, "Sedimentation and Its Relation to Oil Finding"; and Dr. Walter H. Bucher, professor of geology, Columbia University, "The Deformation of the Earth's Crust."

Except for Major Moore and Drs. Croneis and Bucher, these speakers were obtained through the Distinguished Lecturers Committee of the American Association of Petroleum Geologists.

Attendance at the first six meetings, each held in Pittsburgh, has averaged slightly more than a hundred persons. As has been hoped for by the organizers, the drawing range extends into Ohio and West Virginia as well as throughout western Pennsylvania. The cooperation of personnel of the U. S. Geological Survey and of the Pennsylvania and West Virginia geological survey groups has been of much assistance. It is planned to hold monthly meetings except for the summer months, these to be in Pittsburgh. Field conferences may be held when transportation facilities are improved.

The diverse geologic interests represented in this area include petroleum and gas, coal, iron and steel, refractories and other ceramic industries, as well as academic groups and amateur geologists.

The next meeting is scheduled for 7:30 P.M., May 28, in the club rooms of the Engineers Society of Western Pennsylvania in the Hotel William Penn. This will be a symposium on the wartime activity of the U. S. Geological Survey in the Ohio-West Virginia-Pennsylvania area, particularly concerned with detailed study of the Berea sandstone. The discussion will be led by Dr. James F. Pepper, David F. Demarest and Dr. Gordon Rittenhouse.

Further information concerning the society and its activities may be obtained by addressing W. B. Robinson, Gulf Research and Development Company, Box 2038, Pittsburgh 30, Pa.

THE EFFECT OF NUTRITION ON THE COURSE OF TUBERCULOSIS

THE Hastings Foundation has purchased a tract of land from the La Vina Sanatorium and is converting a former preventorium building into the Charles Cook Hastings Home. The site is just north of Pasadena, Calif. Construction is nearing completion, and Dr. Edward Kupka, medical director of both institutions, has announced that the opening day will be about August 1. The Hastings Home is being established pursuant to the will of Charles H. Hastings, who left approximately three million dollars for the study and treatment of tuberculosis. The institution is to be a research type hospital in memory of his father, who died of the disease.

Agreement has been made with the Veterans' Facility, San Fernando, to treat twenty veterans of this war who have developed tuberculosis. The entire cost of their care will be paid by the Hastings Foundation.

Dr. Horace R. Getz, research associate of the Henry Phipps Institute of Philadelphia, will be director of the laboratories. He will continue the research on nutrition hitherto carried on by him in Philadelphia.

An attempt will be made to learn the effect of nutrition on the course of tuberculosis. It is planned to study minimal cases only at first. Objective nutrition tests on the blood and biomicroscopic examination of the eyes, tongue and gums will be a part of the routine examination of the subjects, all of whom will receive a good standard sanatorium diet with one half being given food supplements. Progress of the disease in both groups will then be carefully compared.

AFFILIATION OF SIGMA ALPHA WITH ALPHA EPSILON DELTA

SIGMA ALPHA, premedical fraternity of the University of Louisville, on April 29 became affiliated with Alpha Epsilon Delta, national honorary premedical fraternity, upon their installation as the Kentucky Alpha chapter. The initiation of eighteen active and three honorary members was conducted by Dr. Hugh E. Setterfield, associate professor of anatomy at the School of Medicine of the Ohio State University and national president of Alpha Epsilon Delta. Sigma Alpha was organized on March 29, 1933, at Louisville, under the auspices of Dr. A. W. Homberger, head of the department of chemistry and premedical adviser. Their affiliation marks another step in the activities of Alpha Epsilon Delta.

Founded at the University of Alabama in 1926 by a group of premedical students, Alpha Epsilon Delta has expanded its membership to include thirty-four

chapters, all at institutions recognized by the Council on Medical Education and Hospitals of the American Medical Association. The object of the fraternity is to encourage excellence in premedical scholarship, to stimulate an appreciation of the importance of premedical education in the study of medicine, to promote cooperation and contacts between medical men and premedical students and educators in developing an adequate program of premedical education and to bridge the gap between the premedical and medical schools.

In addition to an extensive program of chapter activities, the fraternity is actively engaged in a number of projects pertaining to premedical education. It sponsored a symposium on premedical education at the September, 1944, meeting of the American Association for the Advancement of Science which led to plans for further meetings with other national and regional organizations interested in medical and premedical education. At the present time, it is conducting a survey of opinion among medical and premedical educators to determine the requirements for an improved postwar program of premedical education.

Honorary members of the charter group are Dr. Alfred Wm. Homberger, professor of physiological chemistry, School of Medicine; Dr. Harvey B. Lovell, acting head of the department of biology, and Dr. Clarence C. Vernon, acting head of the department of chemistry, who will serve as faculty adviser for the chapter.

Dr. Setterfield gave the main address at the installation banquet, entitled "What Kind of Doctor?" He stressed that a broad liberal college education is the best preparation for the study of medicine and said that the technical training afforded by specialized courses is needed, but that these should be kept to a minimum consistent with successful accomplishment in the medical school. The importance of a wide understanding of the social problems of individuals and communities in relation to medicine was emphasized, and he urged that these problems be recognized as some of the most basic needs in the medical profession of the future.

A number of guests and speakers were in attendance at the installation banquet, including Dr. E. W. Jackson, president of the University of Louisville, and Dr. J. J. Oppenheimer, dean of the College of Liberal Arts.

SCIENTIFIC NOTES AND NEWS

THE presentation of the third annual chemurgy award of the United States Chamber of Commerce was made recently at a special luncheon in Omaha, Nebr. The award this year was given to the University of Nebraska as a result of research by a group of scientific men headed by Dr. Eric Kneen, associate agricultural chemist at the university, who accepted it on behalf of his associates and himself. Also receiving special mention was Dr. R. M. Sandstedt, professor of agricultural chemistry.

THE King of Norway has conferred the Order of St. Olaf, First Class, on Dr. J. C. Geiger, director of Public Health of the City and County of San Francisco. The decoration carries with it an honorary knighthood and the following citation: "For valuable and distinguished service to Norway in World War II, and for unswerving devotion and intelligent administration of public health in order that human lives be preserved and the world made a better and happier place to live." The order was established in 1847 by Oscar I in honor of St. Olaf, the founder of Christianity in Norway.

DR. AARON J. SHARP, associate professor of botany at the University of Tennessee, has been elected a member of the Sociedad Mexicana de Historia Natural and of the Sociedad Botanica de México. Under a Guggenheim fellowship, Dr. Sharp is studying the relationships between the vegetation of the Southern Appalachians and the highlands of Mexico and Central America.

THE Finney-Howell Research Foundation, Inc., has awarded fellowships as follows: Muriel Virginia Bradley, Ph.D. (California), to work in the Radiation Laboratory of the University of California, second award; Margaret Aston Kelsall, Ph.D. (Colorado), to work at the Jackson Memorial Laboratory, Bar Harbor, Maine, second award; Nelicia Maier, Ph.D., M.D., the Sorbonne, Paris, and Medical School, Paris, to work with Dr. Rhoads at the Memorial Hospital of New York City, third award; Elizabeth Cavert Miller, M.S. (Wisconsin), to work in the department of biochemistry of the University of Wisconsin, first award.

DR. ROSS ALLEN BAKER, professor of chemistry in the School of Business and Civic Administration of the College of the City of New York, was elected chairman of the New York Section of the American Chemical Society at the annual meeting of the section, on May 12. He succeeds Dr. Beverley L. Clarke, head of the analytical department of the Bell Telephone Laboratories.

BECAUSE of restrictions due to the war emergency, the membership of the Association of Southeastern Biologists has voted not to hold the annual meeting

this spring and to retain the incumbent officers. These are: *President,* Dr. Mary Stuart MacDougall, Agnes Scott College; *President-elect,* Dr. James T. Penney, University of South Carolina; *Vice-president,* Dr. Reed O. Christenson, Alabama Polytechnic Institute; *Secretary-Treasurer,* Dr. Martin D. Young, National Institute of Health, U. S. Public Health Service.

DR. LAWRENCE M. GOULD, professor of geology and geography at Carlton College, Northfield, Minn., has been made president of the college.

DEAN A. A. POTTER, of the School of Engineering, has been made acting president of Purdue University.

DR. ABRAHAM CANTAROW, associate professor of medicine at the Jefferson Medical College, Philadelphia, has been appointed professor of physiological chemistry to succeed Dr. George Russell Bancroft, who will retire in June.

AT the University of London, Professor Harold Davenport, F.R.S., of the department of mathematics at University College, Bangor, has been appointed Astor professor of mathematics, and Dr. Edmund Giffen, since 1940 director of research at the Institution of Automobile Engineers, has been appointed to the university chair of civil and mechanical engineering tenable at Queen Mary College.

DUDLEY E. CHAMBERS, assistant engineer of the Division of Transmitter Engineering of the General Electric Company, has been appointed executive engineer of the Research Laboratory in Schenectady. He succeeds Dr. Laurence A. Hawkins, who has held the post since it was established in 1912 and who has now retired, though remaining on the staff as consultant. The executive engineer is business manager of the laboratory in charge of coordinating the varied research work of its staff with the engineering activities of the company.

DR. ROBERT N. DU PUIS and Dr. C. S. Miner, Jr., of the Miner Laboratories in Chicago, have been made assistant directors of research in the laboratories.

DR. NELSON W. TAYLOR, of the Minnesota Mining and Manufacturing Company, has become technical assistant to the director of research in addition to his present position as head of the Section of Ceramics. He was formerly head of the department of ceramics at the Pennsylvania State College.

M. HERBERT EISENHART, president of the Bausch and Lomb Optical Company, has been elected chairman of the board of trustees of the University of Rochester.

A. O. RHOAD, superintendent in charge of the Iberia Livestock Experiment Farm, Jeanerette, La., has been appointed, beginning on July 1, chief of the Division of Animal Industry of the Inter-American Institute of Agricultural Sciences at Turrialba, Costa Rica.

COLONEL CLIFFORD C. GREGG, director of the Chicago Natural History Museum, who has had leave of absence for several years for service with the Army, has returned to his post at the museum and is on terminal leave until September. In his absence Orr Goodson has been acting director, serving in that capacity until April 30.

A CORRESPONDENT writes: "Readers of SCIENCE may be interested to hear that word has been received from Madame Irene Joliot-Curie that she and her family are well, though they have passed through many dangers. The Curie laboratories have not been injured and she is still working there. Her husband, Professor Frederic Joliot-Curie, was president of the 'Front National' and now is director of the Center of Scientific Research. They hope to visit the United States at some time in the future, as they have been for so long without communication with our universities."

THE annual Herter lectures at the New York University College of Medicine were given on May 22 and 23 by Dr. Peyton Rous, of the Rockefeller Institute for Medical Research, on "The Search for the Activating Causes of Cancer."

DR. OTTO LOEWI, recipient of the Nobel Prize in Medicine in 1936, will deliver an address at Hunter College under the auspices of the Department of Physiology and Hygiene, on May 31 at 4:00 P.M. The department is celebrating the twenty-fifth anniversary of the establishment of a major in physiology. The title of the lecture is "From the Workshop of Discoveries."

MAJOR SAMUEL R. M. REYNOLDS, A.U.S., who is on leave of absence from the Department of Embryology of the Carnegie Institution of Washington, addressed on May 8 the Section of the Society of Automotive Engineers of the District of Columbia. The subject of the address was "Human Engineering in the Army Air Forces."

PROFESSOR RICHARD FOSTER FLINT, on leave from Yale University for duty in the Army Air Forces, addressed on May 10 a meeting of the Southeastern Geological Society at Tallahassee, Fla. His subject was "Pleistocene Seas in North America."

DR. FULLER ALBRIGHT, of the Harvard Medical School, spoke at the April meeting of the Osler Clinical Society at the College of Medicine of the University of Vermont on "Ovarian Function and Dysfunction."

WANDA K. FARR, of the Research Division of the

Celanese Corporation of America, gave on the evening of May 25 a lecture before the New York Chapter of the American Institute of Chemists on "The Utilization of Plant Cell Membranes."

ON recommendation of the War Policy Committee, the president and secretary of the American Mathematical Society presented to the War Committee on Conventions an application for permission to hold the June 24 and 25 meeting in Montreal at the close of the Canadian Mathematical Congress. The application has been denied. Consequently, with the approval of the council, the meeting has been cancelled. At its meeting on April 28, the council authorized the president and secretary to arrange for a summer meeting in September, 1945, if war conditions permit a relaxation of the regulations of the Office of Defense Transportation.

A CONFERENCE on the "Problems of the Premedical College in Fitting the Physician for His Place in Modern Society" was held on April 20 and 21 at Union College, Schenectady.

AN Anesthesia Essay Contest sponsored by the American Society of Anesthesiologists, Inc., offers a prize of $100 for the best original essay, not published previously, on some phase of anesthesiology or on a subject closely related to it. Information concerning the contest may be obtained by writing either to Dr. R. Charles Adams, Mayo Clinic, Rochester, Minn., or to Dr. McKinnie L. Phelps, 745 Fifth Avenue, New York 22, N. Y.

THE question of an appropriation for postwar building for the University of Illinois is now before the legislature. Among the buildings which have been requested is one for chemistry and chemical engineering, the cost of which is estimated at $1,700,000.

THE program of research education of the Battelle Memorial Institute, Columbus, Ohio, which was curtailed owing to war conditions, will be expanded under the direction of Dr. J. Robert Van Pelt, who recently joined the staff of the institute. Fellowships will be available to highly qualified graduate students. These fellowships, which hitherto have been restricted to students at the Ohio State University, will now be made available in a number of institutions of higher learning.

THE University of Chicago has received a grant of $175,000 for research in pediatrics from the estate of the late Dr. Walter H. O. Hoffmann.

GIFTS amounting to over $50,000 have been received by Columbia University. Fifteen thousand dollars has been appropriated by the Josiah Macy Jr. Foundation for the research program on aging in the School of Medicine; $9,000 by the Corn Industries Research Foundation, and $8,000 by the Rockefeller Foundation. The Viking Fund appropriated $6,000 to bring Dr. Francis L. K. Hsu, of the National Yunnan University, China, to carry on research in the department of anthropology.

SUBSIDIES of $8,000 to consolidate gains made in safety engineering education during the war have been made by the National Safety Council to New York University, the Georgia School of Technology, the University of California and the Illinois Institute of Technology.

DR. GHENT GRAVES, Mrs. George T. Morse and Mrs. Laura Lee Graves Steel, of Houston, Texas, have given funds to the Medical Branch at Galveston of the University of Texas to establish a research fellowship in internal medicine in honor of their father, Dr. Marvin Lee Graves, emeritus professor of medicine in the School of Medicine.

FOR the third successive year the W. K. Kellogg Foundation has made a grant to the Pan American Sanitary Bureau in support of a nutrition project in Mexico conducted by the Nutritional Biochemistry Laboratories of the Massachusetts Institute of Technology in collaboration with the Mexican Institute of Nutrition. In previous years, three physicians and two chemists from Mexico were trained in this country; a large number of Mexican foods were analyzed; a federal school lunch program was initiated; the nutritional status of a thousand school children in Mexico City was determined, and the equipment for a modern food analysis laboratory was provided. A part of the present grant of $10,300 will be used to encourage the further analysis of Mexican foods. Dr. Francisco de P. Miranda is chief of the Mexican Institute of Nutrition, and Professor Robert S. Harris, of the Massachusetts Institute of Technology, is in charge of the project.

IT is reported in *Nature* that the Club for Research on Aging of the department of zoology and comparative anatomy of the University of Oxford has received from Lord Nuffield a gift of £3,000 to enable Dr. V. Korenchevsky to continue his gerontological investigations for a period of three years.

IT is stated in *The Times*, London, that the Imperial College of Science and Technology, with the approval of the Court of the University of London, has been presented by Courtaulds, Ltd., with a fund calculated to yield an income of £3,000 a year in perpetuity. It will be administered for the present by a small body of trustees, and will permit the institution of a Courtaulds chair of chemical engineering, to which it is expected that an appointment will be made this year. It will also provide for other needs in the department of chemical technology.

DISCUSSION

THE PULSATION THEORY OF STELLAR VARIATION

ALTHOUGH pulsation of stars had been suggested some years earlier, its recognition as a probable cause of stellar variation dates from the demonstration by Shapley[1] that the dimensions of Cepheid variable stars were incompatible with an explanation in terms of orbital motion. Eddington[2] developed the mathematical theory, but a serious disagreement with observation was found. The theory indicated that the star should be at light maximum when it was smallest and at minimum when largest, since change of temperature would much more than offset change of size. The radial velocities, however, showed most rapid approach of the stellar surface (most rapid expansion) at light maximum and most rapid collapse at light minimum. In spite of the contradiction, the pulsation theory enjoyed wide acceptance and astronomers hoped that the difficulty would eventually be removed.

In the following discussion, in agreement with the conventional method of plotting the radial velocity curve, we shall use "velocity minimum" to mean greatest velocity of approach and "velocity maximum" to mean most rapid recession. On the pulsation theory, these two phases are most rapid expansion and most rapid collapse, respectively.

Eddington's solution corresponds to a standing wave throughout the star. The theory was modified by M. Schwarzschild,[3] who introduced the postulate of running waves in the outer less dense layers, while the standing wave solution was retained for the interior. For the type star, Delta Cephei,[4] the Schwarzschild theory gave excellent agreement with the observed correlation of light and velocity curves.

For the long-period variable star Mira (Omicron Ceti)[5] the velocity curve based on the absorption lines has its maximum at light maximum, just the opposite of the relation in Cepheids, and also in serious conflict with the older pulsation theory. The radiometric observations[6] by Pettit and Nicholson yield a computed velocity curve almost one half period out of phase with the observed absorption velocities.

The anomalous relative strengths of the bright hydrogen lines of long-period variables had long been recognized as an indication that the absorption may occur at much higher atmospheric levels than the bright lines. R. M. Scott[7] showed that the integration of the velocity curve of the bright lines of Mira gave a variation of the star's diameter that was in good agreement with the radiometric data and with the variation to be expected according to the Schwarzschild theory, since the minimum of emission velocity comes a short time later than maximum light. The absorption velocity lags about 150 days (about 0.4 period), which represents the time of travel of the running wave from the effective emitting to the effective absorbing layer.

We shall now show how several other observed relationships find a logical explanation in the running wave pulsation theory.

In 1923 at the University of Michigan Observatory, J. A. Aldrich[8] found that when absorption lines in the spectrum of the Cepheid variable S Sagittae were segregated according to the level at which the lines occurred in the solar chromosphere, the phases of the velocity curves of higher levels lagged relatively to the lower. The effect was soon found in Eta Aquilae[9] by Rufus and in W Sagittarii[10] by Curtiss. Similar effects have since been found in several other Cepheid variables, chiefly by observers at Michigan.[11] In this lag of phase of the velocity curves of higher atmospheric levels, we have clear observational evidence of the reality of the running waves which emerge from the stellar photosphere at light maximum and pass upward through the atmosphere.

The writer[12] pointed out that Cepheids, semiregular variables of intermediate period and long-period variables exhibit a "period-lag" relation. In the five-day Cepheids the velocity minimum is simultaneous with light maximum, but in Cepheids of longer period the velocity minimum comes about one tenth period later. This lag increases through the semi-regular stars, and in the long-period variable Mira (period 330 days) it is so great that velocity minimum comes at light *minimum*. Confirmation of the lag among the Cepheids was found by Joy[13] from very extensive observational material. The RV Tauri stars[14] as a class show a lag that is comparable to that of the Cepheids of longest period (about 45 days). The most regular example is AC Herculis.[15] The sequence continues

[1] H. Shapley, *Astrophys. Jour.*, 40: 448, 1914.
[2] A. S. Eddington, *Monthly Notices, Royal Astron. Soc.*, 79: 2, 1918; 177, 1919; 87: 539, 1927.
[3] M. Schwarzschild, *Circulars, Harvard Observatory*, No. 429, 1938.
[4] M. Schwarzschild, *Circulars, Harvard Observatory*, No. 431, 1938.
[5] A. H. Joy, *Astrophys. Jour.*, 63: 281, 1926.
[6] E. Pettit and S. B. Nicholson, *Astrophys. Jour.*, 78: 320, 1933.
[7] R. M. Scott, *Astrophys. Jour.*, 95: 58, 1942.
[8] J. A. Aldrich, *Popular Astronomy*, 32: 218, 1924; *Publ. Observatory, Univ. of Mich.*, 4: 75, 1932.
[9] W. C. Rufus, *Proc. Nat. Acad. Sci.*, 10: 264, 1924; *Publ. Observatory, Univ. of Mich.*, 4: 101, 1932.
[10] R. H. Curtiss, *Publ. Ast. Soc. of the Pacific*, 38: 148, 1926.
[11] See several papers in *Publ. Observatory, Univ. of Mich.*, Vols. 4, 5 and 6, by Rufus, D. W. Lee and R. M. Petrie.
[12] D. B. McLaughlin, *Astron. Jour.*, 40: 16, 1929.
[13] A. H. Joy, *Astrophys. Jour.*, 86: 363, 1937.
[14] D. B. McLaughlin, *Astrophys. Jour.*, 94: 94, 1941.
[15] R. F. Sanford, *Astrophys. Jour.*, 73: 364, 1931.

through R Scuti[16] (an RV Tauri variable with period 140 days), SX Herculis[17] and R Virginis[18] and ends with Mira.

The period-lag relation can be explained in terms of increased time of travel of the compressional wave from the photosphere to the effective absorbing layer. In five-day Cepheids the absorption takes place close to the photosphere; in long-period variables it occurs possibly 8×10^7 kilometers above the photosphere. In a series of stars from the five-day Cepheids to the long-period variables, the radii of the stars increase from about 20 to 500 times that of the sun, and the acceleration due to gravity at the photosphere decreases from about 500 to 2 cm/sec^2. Correspondingly lower density and greater volume of atmosphere is to be expected about the larger stars.

From measures made near the time of light maximum in the spectra of a large number of long-period variables, Merrill[19] established a relation between the period and the amount of displacement of the emission lines towards the violet relative to the absorption spectra. With increased period of variation, the emission shows an increased difference of radial velocity, in the sense that it is approaching, relatively to the absorption. This effect also finds a plausible explanation in terms of the running wave theory and the Scott model of a long-period variable with the emitting layer far below the absorbing layer. In the long-period variables of shortest period (about 120 days), the distance between the emitting and absorbing layers is relatively small; the travel-time of the wave, the difference of phase, and hence the difference of velocity, will also be small at any instant. For the larger stars of longer period, the difference of phase and hence the difference of velocity at light maximum will increase. This is because the minimum of emission velocity comes shortly after maximum light, but the minimum of absorption velocity is delayed long after maximum. A fluctuation of the correlation near a period of 250 days remains unexplained.

The radiometric measures by Pettit and Nicholson and the infra-red photographic observations by Hetzler[20] showed a marked lag of the date of light maximum of long-period variables for long-wave radiation as compared with the curves for visible light. A similar effect has just been announced by Stebbins[21] for Delta Cephei; light curves measured in six wave-lengths show a progressive delay of maximum for the longer wave-lengths. These observations are entirely compatible with the present pulsation theory, at least qualitatively. At light maximum and for more than a quarter of its period thereafter, the variable star is expanding. After maximum it is cooling. Two opposing tendencies are then in operation: expansion tends to increase the brightness, but cooling tends to decrease it. The cooling is the more effective in all wave-lengths, so that the star fades. According to the Planckian law, the cooling causes the most rapid fading in the shorter wave-lengths; this has the effect of making the maximum earliest in the short wave-lengths, latest in the long.

The more detailed explanation of the forms of light and radial velocity curves of different variables is not so readily forthcoming. But it can now be said that the pulsation theory of stellar variation has reached such a stage of development and of agreement with observation that we no longer need apologize for its remaining shortcomings.

DEAN B. MCLAUGHLIN
CAMBRIDGE, MASSACHUSETTS
(On leave from the University of Michigan)

[16] D. B. McLaughlin, *Publ. Observatory, Univ. of Mich.*, 7: 57, 1938.
[17] A. H. Joy, *Astrophys. Jour.*, 75: 127, 1932.
[18] P. W. Merrill and A. H. Joy, *Astrophys. Jour.*, 69: 379, 1929.
[19] P. W. Merrill, *Astrophys. Jour.*, 93: 383, 1941.
[20] C. W. Hetzler, *Astrophys. Jour.*, 83: 372, 1936.
[21] J. Stebbins, *Astrophys. Jour.*, 101: 47, 1945.

THE PRESENCE OF ALLOXAN IN NORMAL LIVERS

ALLOXAN is readily prepared *in vitro* by the action of certain oxidizing agents on uric acid; since it is known to cause experimental diabetes in animals it was of interest to examine the organs of normal animals for its possible presence. To date, the only such observations, made many years ago, appear to be its detection in the gelatinous mucus from an intestinal catarrh[1] and in the urine of a heart patient.[2] In the vegetable kingdom it has been found (as the "half-reduced" form, alloxantin) in the hydrolysates from crude beet juice,[3] broad (fava) beans and vetch seeds.[4]

A test which has been generally accepted for the identification of alloxan is based on the fact that on stepwise reduction it gives first alloxantin (a 1:1 compound of alloxan and dialuric acid) and then dialuric acid; if barium hydroxide is added to this reduction product, a purple color or purple precipitate is formed. Unfortunately, in the form described in the literature, this test is not well suited to the study of biological materials. We have now devised conditions under which, using as little as 1 cc of 0.01 per cent. alloxan monohydrate solution, this purple color is readily detectable by the naked eye; we have employed ascorbic acid or, preferably, cysteine hydrochloride as the reducing agent.

We next proceeded to examine a variety of animal

[1] J. von Liebig, *Ann.*, 121: 80, 1862.
[2] G. Lang, *Z. anal. Chem.*, 6: 294, 1867.
[3] E. O. von Lippmann, *Ber.*, 29: 2645, 1896.
[4] H. Ritthausen, *Ber.*, 29: 894, 2106, 1896; H. J. Fisher and T. B. Johnson, *Jour. Am. Chem. Soc.*, 54: 2038, 1932.

and vegetable tissues and fluids; solid materials were extracted with 1 part of 10 per cent. trichloracetic acid by maceration or in the Waring Blendor; liquids were treated with one volume of the acid solution. A sample of the filtrate was then decolorized by shaking with charcoal, again filtered, and tested as described above.

We wish to report at this time that the purple color mentioned was obtained with such extracts of fresh, normal animal livers (beef, cat, calf, domestic fowl, guinea pig, lamb, rabbit and rat), suggesting the presence of alloxan in these materials. Experiments designed to test the validity of this tentative conclusion are in progress. However, we are disposed to believe that formation of the purple color is associated with the presence of alloxan for the following reason: the blood of normal, untreated rabbits does not respond to the color test, but the blood of treated rabbits (which have received alloxan in the alimentary canal) gives the color test at 5 minutes and at 2 hours after commencing administration. The purple color was not obtained with the fresh livers of shad, or sea perch ("croaker" fish).

Precise details for performing the test, observations made on other animal tissues and fluids, and the bearing of these results on the diabetes problem will be discussed in a forthcoming communication.

J. A. RUBEN

THE MONTEFIORE HOSPITAL

R. STUART TIPSON

THE DEPARTMENT OF RESEARCH IN PURE CHEMISTRY,
MELLON INSTITUTE

ACHIEVING FULL EMPLOYMENT AFTER THE WAR

DR. MAYER'S recent article on how to achieve and maintain full employment after the war[1] deals ably with the problem of organizational approach to the immediate situation but fails to indicate how important *research* can be in building the foundations for a healthy and expanding economic order. This is an idea of great interest to scientists, but it has not been widely appreciated. Research is seldom stressed in the discussion of postwar plans.

Charles F. Kettering has said, "if we could get out of our minds the idea that we know a lot about everything and realize that the whole thing [research] is ahead of us, then we would have a shortage of labor in no time." The number of people directly concerned with research could be greatly increased, resulting in increased employment, but more important than this direct use of manpower, research creates new industries and new outlets for man's endeavors which furnish the basis for widespread employment.

Scientific research has been a very minor item of expense and effort in the life of Americans. Probably not more than one person in a thousand is engaged in research. Compared with what we put into war, research has been developed only to microscopic proportions. Yet it is trite to say that enormous industries—telephone, radio, electrical, aeronautical, glass, synthetic fibers and plastics, photographic, moving picture, dyestuff and food—all these and more have their foundations in the relatively small amount of scientific research which has been promoted.

The problem of unemployment is not merely that of finding something for people to do—it is finding *profitable* and *useful* pursuits. What holds more promise directly or indirectly than research? Research can delve into numerous fields and can unearth more treasures than the world dreams of. Every existing industry and means of livelihood will yield profitable findings, and on top of all this and perhaps fully as important, research about ourselves can furnish the key to vastly better health and adjustments—mental as well as physical.

From the standpoint of national safety research is second to nothing in importance. Our ability to hold our place and prevent war will depend more than anything else upon the strength of our science and technology—nothing whatever can take its place. Unfortunately the current handling of our manpower problem has been such as to leave us very shorthanded with respect to scientists in the postwar development ahead. For defense reasons, if for no other, we can afford to devote our efforts to a greatly expanded research program.

One method by which it should be possible to develop and encourage the research which must be at the basis of much of our future development would be to give corporations and individuals generous tax exemptions based upon the amounts which are spent on research. I am sure that many who have had experience in research will agree that a substantial amount of investigation should remain in private hands and that government-sponsored research is liable to become involved in red tape and organization to such an extent that it becomes sterile or nearly so. Research must be in the hands of competent leaders who can be replaced as needed by younger and more vigorous men.

If we should spend ten times as much for research as we do, it would be only a fraction of what we will spend for national defense, yet we would be laying a foundation for vast employment for many years to come.

ROGER J. WILLIAMS

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[1] J. Mayer, SCIENCE, 101: 367, 1945.

SCIENTIFIC BOOKS

THE MARIHUANA PROBLEM

The Marihuana Problem in the City of New York; Sociological, Medical and Pharmacological Studies. By the MAYOR'S COMMITTEE ON MARIHUANA. 220 pp. Lancaster, Pa.: The Jaques Cattell Press. 1944. $2.50.

THIS report includes a thorough study of the ancient hashish vice as it currently exists in New York City. Primarily intended as a local project, the findings have considerable value of a general nature. The publication is a close parallel to the "Indian Hemp Drugs Commission Report" sponsored by the British Government in India about fifty years ago. Together these two studies represent the most significant of Government instigated inquiries into the sociological aspects of this drug problem. In both cases, several official investigators were commissioned to spend a year or more collecting cross-sections of testimony and opinions from individuals who had practiced this drug habit or had observed its effects at close range. The earlier report was relatively massive in comparison (3,281 pages in 7 volumes). The current study gives no direct indication of familiarity with this earlier report and obviously was not materially influenced by the earlier conclusions. This feature gives greater reliability to the findings of both, which were reasonably similar despite the wide differences in locale. In the case of both surveys it was obvious to the sponsors that a satisfactory understanding of the public effects of this practice could be reached only by an associated clinical and pharmacological inquiry. As with the first study, the scientific work included in the New York report represents a substantial contribution to existing clinical and pharmacological knowledge of the subject. Earlier impressions with sporadic measurements are now supported by concerted observations using the methods of the modern clinical laboratory. Electroencephalograms, electrocardiograms, blood chemistry, roentgenograms, expertly devised psychological tests, Rohrschach interpretations, etc., were carried out in a group of seventy-two subjects, forty-eight of whom gave a history of marihuana smoking and all of whom were drawn from the social level in which this drug vice is most likely to appear. The conditions of this study, as carried out at Welfare Hospital, afforded a unique opportunity for standardization and control. The acute episodes showed no significant physiological changes beyond those attributable to the characteristic emotional instability. The old observation of hunger and a desire for sweets was frequently observed but not explained by the metabolic studies; blood sugars commonly, but not consistently, showed a moderate rise. Frequent micturition, another old observation, was not associated with any significant diuresis. Of the psychomotor functions, body and hand steadiness and complex reaction time were most adversely affected. Auditory acuity, musical ability and estimations of time and space were not significantly affected as judged objectively; subjectively, as previously described, these functions were thought to be disturbed. Psychological observations indicated increased feelings of relaxation, disinhibition and self-confidence, which latter was expressed primarily through oral rather than physical activity. The basic personality structure was not changed.

Conclusions, previously recognized but now better supported with these studies, are to the effect that marihuana smoking, at least as ordinarily practiced in this country, does not lead directly to mental or physical deterioration, does not develop addiction or tolerance as is characteristic of opiates, and is not a direct causal factor in sexual or criminal misconduct. As is well known, a number of serious, qualified observers in various parts of the world, including this country, have been somewhat more inclined to favor a conception of true marihuana addiction and to ascribe a causal relation of marihuana to physical and mental deterioration and to criminal behavior. Supporting their views is the fact that they have been able to observe cases where the vice has existed for a much longer time and under conditions where the subjects might be more likely to follow criminal impulses. With due consideration to the possible correctness of some of these observations, this survey in New York City is convincingly sound within the field it attempts to cover. It may be a point of significance that the history of this drug records only three prominent survey projects conducted by groups of physicians and scientists, and in each case the findings have tended to minimize the gravity of the marihuana problem. The reference is to the Indian Hemp Drugs Commission Report in 1893, the report of six military physicians and health officers in Panama in 1933 and this present survey by a group of about thirty individuals, the majority of whom were physicians.

In New York City, according to the report, marihuana smoking is chiefly confined to Negroes and Latin-Americans living in the Harlem area, where this survey estimates there are about five hundred "teapads" and at least five hundred peddlers. The cost is low and there are no potent, well-organized syndicates engaged in the traffic. Among school children over the city at large, the practice has proved to be a minor disciplinary problem in isolated instances. This latter observation probably reflects the situation over the United States as a whole, where school-yard abuse was a serious problem only in the interval between introduction of the vice and the passage of adequate laws restricting the sale of marihuana.

The strictly pharmacological studies reported by S. Loewe record for the first time a full account of the biological control work associated with the chemical identification and synthesis of active compounds as carried out at the University of Illinois and the Bureau of Narcotics Laboratory. This highly successful example of teamwork has furnished new starting points for future inquiries. Some valuable drugs may subsequently be developed from findings of this sort and the problem deserves further study beyond the obviously unpromising attempt to use marihuana compounds in alleviating the withdrawal symptoms of morphine addiction.

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REPORTS

GUGGENHEIM FELLOWSHIP AWARDS

THE award of ninety-six fellowships and $230,000 to the recipients is announced by the John Simon Guggenheim Memorial Foundation. Forty-one of the newly appointed fellows received their awards under the plan for post-service fellowships available to Americans who are engaged directly in the war effort in the Army, Navy and civilian war agencies. Their fellowships will be held for their use after they are discharged from service. The foundation will make another series of appointments to post-service fellowships in June.

DR. SAMUEL HERRICK, assistant professor of astronomy, University of California, Los Angeles, will carry on studies of navigation. He will investigate its present and prospective methods and instruments and the effect of the introduction of rocket propulsion.

DR. PAUL ERDÖS, mathematician, Ann Arbor, Michigan, will carry out research on polynomials and approximation of continuous functions by polynomials.

Biological studies will be carried on by the following fellows:

DR. CARLOS EUGENIO CHARDON, director of the Institute of Tropical Agriculture, Mayagüez, Puerto Rico: The completion of a historical study of the great naturalists who worked in Latin America. From 1931 to 1935 Dr. Chardon was chancellor of the University of Puerto Rico, and he has acted as agricultural adviser to several Latin American countries.

DR. DENIS LLEWELLYN FOX, associate professor of marine biochemistry, Scripps Institution, La Jolla, Calif. The preparation of a monograph on the physical, biological and biochemical features of the structural colors and pigments of animals.

DR. KENNETH WILLARD COOPER, assistant professor of biology, Princeton University: Studies of the mechanism of sex chromosome conjunction during meiosis in male *Drosophila*.

DR. AARON J. SHARP, associate professor of botany, University of Tennessee: Studies of the correlations between the plants of the Southern Appalachians and the temperate floras of the mountains and highlands of Mexico and Central America. This is the second Guggenheim Fellowship awarded to Dr. Sharp, who is now working in Mexico.

DR. DAMON BOYNTON, associate professor of pomology, Cornell University: A study of ion competition as a factor affecting the inorganic nutrition of plants, with particular reference to potassium-magnesium and nitrogen-phosphorus relationships in fruit trees.

DR. THEODORE C. SCHNEIRLA, associate professor of psychology, New York University, and associate curator of animal behavior, American Museum of Natural History: Studies of the relationship between instinct and learning in insect psychology. Dr. Schneirla is now in Tehuantepec, Mexico, where he is studying the behavior of colonies of army ants. This is the second Guggenheim fellowship awarded to Dr. Schneirla.

Awards of fellowships for postwar work in science were made to:

DR. JOHN WILLIAM CALKIN, professor of mathematics, Illinois Institute of Technology, Chicago; now doing war work in Office of Naval Operations, Washington, D. C.: Mathematical studies of the operational theory of boundary value problems and the application of this theory to differential equations.

DR. EDWIN HEWITT, operations analyst, Army Air Forces; formerly instructor in mathematics, Harvard University: Mathematical studies in the field of topology.

DR. LEO LEROY BERANEK, director of research on sound, Harvard University; now engaged upon war research under the Office of Scientific Research and Development: Research in the field of acoustics and design of acoustical materials.

DR. CHANDLER MCCUSKEY BROOKS, associate professor of physiology, the Johns Hopkins School of Medicine; now engaged upon war research under the Office of Scientific Research and Development: A study of the comparative physiology of the nervous and the neuro-endocrine systems.

DR. SEYMOUR STANLEY COHEN, biochemist at the University of Pennsylvania; now engaged upon war research under the Office of Scientific Research and Development: A study of methods of virus and rickettsial cultivation suitable for and preliminary to investigation of biochemical aspects of the host-virus relationship, with special reference to nucleic acid metabolism.

DR. DEAN STANLEY TARBELL, assistant professor of organic chemistry, University of Rochester; now engaged upon war research under the Office of Scientific Research and Development: A study of the synthesis of colchicine and other organic compounds of biological interest.

LIEUTENANT EDWARD NOVITSKI, Army; geneticist, San

Marino, California: Studies of the genetic effects of ultra-high frequency irradiation.

APPRENTICE SEAMAN ELLSWORTH CHARLES DOUGHERTY, specialist training program, Navy; research fellow in zoology, University of California, Berkeley: Studies of the comparative morphology of the parasitic nematodes of the suborder *Strongylina*.

Four fellowships were awarded to Canadian scholars, including two in science, as follows:

DR. ROGER YATE STANIER, penicillin production manager for Merck and Company Ltd., Montreal: Studies on the nature, relationships and biological activities of bacteria, particularly of the myxobacteria.

DR. JOHANNES F. K. HOLTFRETER, department of genetics, McGill University: Investigations of the causal factors involved in the embryonic development of vertebrates. This is the second Guggenheim fellowship awarded to Dr. Holtfreter.

SPECIAL ARTICLES

THE STRUCTURE, FUNCTION AND INHIBITORY ACTION OF PORPHYRINS[1]

Hemophilus influenzae requires hematin for growth. It is unable to synthesize it from ordinary culture media. This fact may be utilized in a study of the function of hematin-like compounds. The results obtained throw some light on the synthesis, action and specificity of various porphyrin compounds. Hoagland and Ward[2] have recently used this organism as an assay method for the second growth factor, coenzyme I, required by this organism. We have utilized their bacteriological procedures in this study.

Hematin is the iron compound of protoporphyrin, which is characterized by its vinyl side chains. Iron-free protoporphyrin supports the growth of *H. influenzae* as well as does hematin,[3] and a positive peroxidase test on organisms grown for several passages on protoporphyrin is evidence for the fact that iron has been inserted into the protoporphyrin ring by the bacillus. Other iron-free porphyrins which contain no vinyl groups, such as hematoporphyrin, mesoporphyrin and coproporphyrin, will not support the growth of the organism. However, the iron compounds of some of these porphyrins, for example, iron mesoporphyrin, will support growth. Therefore it appears that the organism, although it is able to utilize for growth various iron porphyrins, is able to insert iron only into protoporphyrin and that the vinyl groups are required for this operation. The vinyl groups are essential in another respect, namely, for the reduction of nitrate to nitrite. The organisms are able to reduce nitrate in the presence of hemin or of protoporphyrin, but no nitrate is reduced in the presence of other iron porphyrins. Multiplication of the bacillus is unaffected by this inability to reduce nitrate.

Furthermore, some of the iron-free porphyrins actually inhibit the growth-promoting faculty of hematin, protoporphyrin, or other iron porphyrins, which support growth. There is a competitive inhibition between the iron-free porphyrins lacking vinyl groups and the iron porphyrins, over a range of concentrations that is limited by the low solubilities of these compounds. For example, ten molecules of hematoporphyrin inhibit entirely the growth-promoting influence of one molecule of iron protoporphyrin. We regard this fact as evidence for the competition of the various porphyrins for the combination with the specific proteins of the heme enzymes.

There is evidence suggesting that the combination between the specific protein and the porphyrin takes place by the interaction of the ionized propionic acid side chains of the porphyrin, probably with basic groups of the protein. Iron protoporphyrin and protoporphyrin which normally promote growth of *H. influenzae* do not do so if their propionic acid groups are esterified. Also the porphyrins which may act as inhibitors do not inhibit if their propionic acid groups are esterified.

The iron porphyrin proteins are the catalysts which make oxygen available to the organism. If an organism were to produce a porphyrin into which iron could not be inserted this porphyrin could compete with iron porphyrins for the combination with the specific proteins which go to make up the heme enzymes. Such a porphyrin would thus be a natural inhibitor, and in a sense a regulator governing the degree of anaerobic versus aerobic metabolism. A porphyrin of ubiquitous distribution that might function in this manner is coproporphyrin. If the facts derived from *H. influenzae* can be carried over to other organisms, then coproporphyrin under certain conditions might function as a regulator for the rate of oxygen consumption by the cell.

A detailed report on the action of porphyrins on other bacteria and also the effect of metalloporphyrins other than the iron-containing ones, will be published in the near future. We wish to take this opportunity to express our gratefulness to Dr. Leonor Michaelis for his continued interest and advice in these studies.

S. GRANICK
H. GILDER

[1] From the Laboratories of The Rockefeller Institute for Medical Research, New York.

[2] C. L. Hoagland and S. M. Ward, *Jour. Biol. Chem.*, 146: 115, 1942.

[3] M. Lwoff, *Ann. Inst. Pasteur* 51: 707, 1933.

A REACTION OF ASCORBIC ACID WITH α-AMINO ACIDS

DURING the course of an investigation of reactions between ascorbic acid and amines,[1] we observed that α-amino acids reacted with ascorbic acid in the presence of hydrogen peroxide and heat to yield a reddish-colored solution. Ammonia water (U.S.P.) may be substituted for the amino acids. The following α-amino acids were studied and found to be suitable as reagents: asparagine, glycine, leucine, isoleucine, tyrosine, cystine, cysteine, valine, phenylalanine, aspartic acid and dl-alanine. The latter was finally adopted as a standard reagent.

This reaction appears to be specific for ascorbic acid, since it is not given by other reducing substances, ketones, phenols or carbohydrates such as sorbitol, glucose, fructose, mannose, xylose, trehalose, arabinose, starches, d-glucurone, and therefore, it is not based either upon the reducing property of[2] or upon the carbohydrate structure[3] of ascorbic acid.

When this reaction is carried out in aqueous media the sensitivity is limited to solutions containing 50 mg per cent. of ascorbic acid or more, whereas, if the reaction is conducted in glacial acetic acid the sensitivity is increased to 2.5 mg per cent., but the color fades rather rapidly at room temperature.

The employment of dilute copper sulfate solutions instead of H_2O_2 increases the sensitivity of the test in glacial acetic acid media still further. This does not hold for aqueous solutions.

Experimental: The following reagents were used for the detection of ascorbic acid: (a) 5 per cent. dl-alanine dissolved in distilled water; (b) superoxol (Merck) in various dilutions depending upon the approximate amount of ascorbic acid reacting—from 30 per cent. to 0.3 per cent. To each cc of ascorbic acid solution (for spectrophotometric estimation—5 cc) were added 1 cc of 5 per cent. dl-alanine and 0.05 cc of H_2O_2 of various strengths. The test-tubes containing the final solution were placed in boiling water for 2 minutes and were allowed to cool for 15 minutes, and the final colors estimated either visually or spectrophotometrically. (For the test in glacial acetic acid the ascorbic acid was made up in various concentrations in that medium, the alanine reagent in a 1.0 per cent. glacial acetic acid and heated in a water bath for 2 minutes. 0.05 cc of an 0.5 per cent. copper sulfate was used to accelerate the reaction. It was found that the addition of alcohol following heating stabilizes the color.)

For spectrophotometrical determination a total volume of 10 cc were used (5 cc dl-alanine, 0.25 H_2O_2 and varying amounts of ascorbic acid made to volume with distilled water).

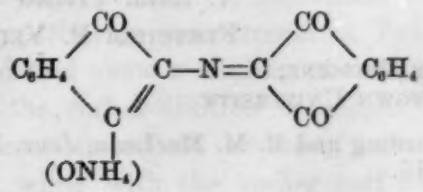
When small amounts of ascorbic acid were used (6 mg/cc or less) the H_2O_2 used was 0.075 per cent. by volume; between 6 and 12 mg/cc ascorbic acid, the final per cent. of H_2O_2 was 0.025, whereas for amounts in excess of 12 mg ascorbic acid, a final per cent. of 0.0075 may be used. With smaller amounts of ascorbic acid and low percentage of H_2O_2 the development of color was slow (may take 15 minutes after boiling); whereas, with larger amounts of ascorbic acid and high percentage of H_2O_2 the color develops during the 2 minutes of boiling. It was found that an excess of hydrogen peroxide will destroy colors produced by small amounts of ascorbic acid and that larger concentrations of hydrogen peroxide were necessary for full development of color using larger amounts of ascorbic acid. The following are the concentrations of peroxide found to be most suitable for maximum color development, using 5 per cent. solution of alanine throughout: between 1–6 mg ascorbic acid per cc—3.0 per cent. H_2O_2; between 6–12 mg ascorbic acid per cc—3.0 per cent., and beyond 12 mg ascorbic acid per cc—10 per cent. of H_2O_2 (0.025 cc H_2O_2 per cc in all cases).

The color in the test exhibited a maximum absorption between 505–510 mμ in aqueous solution. Upon prolonged standing the color shifts to the yellow end of the spectrum exhibiting a maximum absorption of about 400 mμ. Colorimetric estimation can also be made using these yellow colors.

The reaction is a colorimetric one, that is, within certain ranges the intensity of the red color varies as the concentration of ascorbic acid. Within narrow ranges the test follows Beer's Law; in other words, the K value within these ranges is fairly constant.

Theory: It is well known that the $-N=N-$ group is chromophoric and that the $-NH_2$ or NH_3 groups are necessary for color development in the ninhydrine and murexide reactions. In both cases the reaction involves two molecules of the reagent connected by $-N=H-$ chromophoric grouping. Only compounds containing an α-amino to a carboxyl group give the ninhydrine test. The constitution of the coloring matters resulting from the interaction of $-NH_2$ with ninhydrine, murexide and the possible reaction with the ketone structural formula of dehydroascorbic acid are as follows:

(1) Ninhydrine:

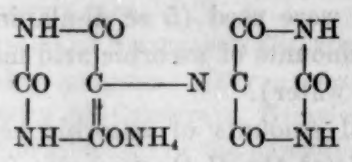


[1] Mr. Louis S. Schwartz, of the Van Patten Pharmaceutical Company, suggested an experimental study of ascorbic acid – NH compounds. We thank him and his firm for the suggestion and for support in carrying out this project.

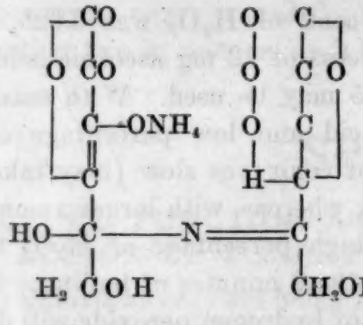
[2] O. A. Bessey and C. G. King, *Jour. Biol. Chem.*, 103: 687, 1933.

[3] J. H. Roe, *Jour. Biol. Chem.*, 116: 609, 1936.

(2) Murexide (Slimmer and Stieglitz formula):



(3) Ascorbic acid (proposed formula):



In support of the theory, it may be argued that in both the ninhydrine and the present test only compounds containing amino group α to a carboxyl are reacting and that we found that in the murexide test the customary reagent, NH_3 water, may be substituted by α-amino compounds (dl-alanine). Also, if dehydroascorbic acid is tested directly with ammonia water and heat, a red color develops without the aid of an oxidizing agent. The facts that the test is more delicate if performed in glacial acetic acid (non-aqueous) media and that copper may be substituted for hydrogen peroxide in the test, indicate that ascorbic acid must be first converted to dehydroascorbic acid to react with $-NH_2$ to yield a colored compound.

It may be stated that the suggestion of Harding and MacLean[4] that 0.2 cc of pyridine to be used for increasing the sensitivity of the ninhydrine test is not applicable to the ascorbic acid reaction because pyridine, along with some other compounds, yields a yellow color in the above procedure which obscures the faint red colors developed.

Conclusions: A new reaction and preliminary colorimetric test of distinctive specificity has been developed by using dl-alanine and H_2O_2 as reagents for the detection of ascorbic acid. It is probable that the color develops with dehydroascorbic acid in a manner similar to the ninhydrine or murexide tests. While the new test may not be fully applicable at the present time to determine ascorbic acid in animal and human materials, it may be used to test the identity of ascorbic acid in official (*e.g.*, U.S.P.) preparations.

We are greatly indebted to Dr. J. H. Roe for checking the reaction and for his encouragement to continue this research.

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[4] V. J. Harding and R. M. MacLean, *Jour. Biol. Chem.*, 20: 217, 1915.

IN VITRO GROWTH AND MULTIPLICATION OF THE MALARIA PARASITE, PLASMODIUM KNOWLESI[1, 2]

WHETHER one is considering the biochemical, biological, immunological or chemotherapeutic aspects of the malaria problem, questions arise which could be more readily answered by experimentation on parasites grown *in vitro* than on those sheltered by the host. This well-recognized fact has led many previous investigators to attempt the *in vitro* cultivation of the malaria parasite. Undoubtedly, the most successful and best-documented work to date on the erythrocytic form has been that of Trager,[3, 4] who clearly demonstrated that the bird malaria parasite *P. lophurae,* would survive *in vitro* up to 16 days at body temperature. Never did Trager observe an increase *in vitro* in the total number of parasites comparable to that seen in the host. The parasite population in his experiments usually remained constant for the first few days and then declined. He concluded that, though development must be continuing in such preparations, the death rate, particularly after the first few days, far exceeds the birth rate.

We wish to report here a brief summary of the results obtained during the past year on the *in vitro* cultivation of the erythrocytic form of the malaria parasite, *P. knowlesi,* in which we have regularly observed growth and good multiplication. *P. knowlesi* was chosen for our work because this parasite has a 24-hour cycle, produces a heavy infection of red cells in the monkey, *Macaca mulatta,* and will also produce infection in man. Moreover, since our chief interest lay in making biochemical and metabolic studies on the parasite, we preferred to deal with a host possessing a non-nucleated red blood cell.

Two types of techniques have been developed. One, which we have termed the rocker-dilution, consists of the dilution of 1 part of whole blood with 3 parts of a nutrient fluid whose composition is given in Table 1. This mixture, usually 6.0 ml in total volume though 50 ml have been used, is placed in a tube or flask equipped with gas inlet and outlet tubes. The container is then placed on a rocking machine which just keeps the red cells in gentle motion. A slow flow of 5 per cent. CO_2: 95 per cent. air is passed into the vessel without being allowed to bubble through the liquid. The whole procedure is carried out under sterile conditions and the cultivation performed at

[1] From the Department of Biological Chemistry, Harvard Medical School, and the Department of Comparative Pathology and Tropical Medicine, Harvard School of Public Health and Harvard Medical School, Boston.

[2] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the President and Fellows of Harvard College.

[3] W. Trager, *Jour. Exp. Med.*, 74: 441, 1941.

[4] W. Trager, *Jour. Exp. Med.*, 77: 411, 1943.

TABLE 1

COMPONENTS OF NUTRIENT MEDIUM*

	gm/L		microgm/L
$MgCl_2$	.095	Adenine Sulfate	250
$CaCl_2$	.056	Guanine-HCl	250
KCl	.410	Thymine	125
NaCl	5.825	Xanthine	250
Na_2HPO_4	.301	Uracil	250
$NaHCO_3$†	2.35	Ascorbic Acid	5000
Glucose	2.50	Biotin	16
Difco Proteose Peptone	1.50	Choline	500
Stearns Amino Acids‡	0.50	Cocarboxylase	400
Glycerol	0.25	Nicotinic Acid	1000
Sodium Acetate	0.15	Nicotinamide	1000
		d-Ca Pantothenate	500
		Pyridoxine	500
		Ribose	500
		Riboflavin	500
		Thiamine	1000

* The freezing point of this medium is $-0.60°$ C. ± .02, and the pH after equilibration with 5 per cent. CO_2: 95 per cent. air is 7.45 ± 0.1.
† Added as Na_2CO_3 and converted to bicarbonate by passing CO_2 gas through the solution.
‡ Fortified with glycine and histidine.

a temperature of 38.5° C. Best results have been obtained when the number of total parasitized cells in the initial mixture is limited to not more than 25,000 per cu.mm., and the total number of red blood cells to 1.25×10^6 per cu.mm. This is usually accomplished by diluting highly parasitized blood with several times its volume of normal blood in order to ensure the presence of an adequate amount of normal blood constituents in the final mixture.

The other technique, which has been termed the rocker-perfusion, employs a Cellophane membrane to separate whole parasitized blood from the nutrient medium. Several types of apparatus for this purpose have been developed. For volumes of blood approximating 1 ml, type 1 is used in which a Cellophane membrane is stretched over one end of a glass tube 30 mm in diameter, and the blood placed within the tube directly upon the Cellophane membrane. This tube is mounted in a vessel containing about 30 ml of nutrient fluid in such a way that the membrane end is just wetted by the nutrient fluid. For volumes of blood approximating 15 ml, type 2 is used in which a coil of Cellophane tubing (8/32 in. dia.) supported by a glass form is immersed in the blood and nutrient fluid allowed to flow through the Cellophane tubing from a reservoir at a rate of approximately 1,500 ml per 24 hours. In both types of apparatus, the container is rocked with a slow stream of 5 per cent. CO_2: 95 per cent. air passing through it. All operations are performed under sterile conditions and cultivation carried out at 38.5° C. As in the rocker-dilution technique, we have usually employed parasitized blood diluted with normal.

Cultivation with all methods is usually carried on for a period of 24 hours. If subculture is performed, then a fresh setup is made each 24 hours, the cultivated parasitized blood being diluted with normal whole blood and the nutrient fluid replaced by fresh.

A summary of the results obtained with the various techniques is given in Table 2. In the 89 experiments given here, the average increase in parasite count within 24 hours has been three- to fourfold. In some sixty monkeys studied to date, the average increase *in vivo* for a similar period and at comparable percentages of parasitization has also been of this order of magnitude.

TABLE 2

SUMMARY OF IN VITRO CULTIVATION EXPERIMENTS IN WHICH TWOFOLD OR BETTER MULTIPLICATION OF PARASITES HAS OCCURRED IN 20–24 HOURS

Method	Total expts.	Number of experiments with the multiplication listed					Avg. multiplication
		2 fold	3 fold	4 fold	5 fold	6–8 fold	
Rocker Dilution	23	2	5	8	5	3	4.0
Rocker Perfusion Type 1	22	3	9	3	3	4	4.0
Rocker Perfusion Type 2	44	19	8	10	4	3	3.2

TABLE 3

DETAILED COUNT ON CULTIVATION EXPERIMENTS RUN BY BOTH TECHNIQUES

Time (hrs.)	0		5		10		24	
Technique*	R.D.	R.P.	R.D.	R.P.	R.D.	R.P.	R.D.	R.P.
Red cells/cmm × 10^6	1.32	3.98	1.30	4.07	..	4.04	1.10	3.78
Parasite count, per cent.	1.8	2.0	2.0	2.2	..	9.2	7.4	9.6
Differential Distribution								
Rings, per cent.	1	1	1	2	..	61	3	12
Trophozoites, per cent.	96	83	29	38	..	31	95	80
Schizonts, per cent.	3	14	69	52	..	3	0	1
Segmenters, per cent.	0	1	0	6	..	2	0	0
Gametocytes, per cent.	0	1	1	2	..	1	2	4
Degenerate and unrecognizable, per cent.	0	0	0	0	..	2	0	3

* R.D. = Rocker Dilution. R.P. = Rocker Perfusion, Type 2. The blood for these two experiments came from two different monkeys so that the similarity in count is merely fortuitous.

In Table 3, a complete differential count for one experiment run by each technique is given. The shift in distribution of the various forms with time is clearly evident, segmentation having occurred sometime between the fifth and tenth hour of *in vitro* cultivation. At the end of 24 hours, the distribution of forms is about the same as at the start of the experiment though four to five times as many parasites are then present. Differential counts for all the 89 experiments summarized in Table 2 are also on record and show a similar picture.

Using the rocker-dilution technique, three successive generations of *P. knowlesi* have been grown *in vitro*, while with the rocker-perfusion (type 1)

technique six successive generations have been obtained. In the latter case, the infectivity of the blood after six days' culture *in vitro* was demonstrated by inoculation into a monkey. It should be pointed out, however, that in order to obtain subculture with the rocker-perfusion technique, it is necessary to replace about one fourth of the nutrient medium with blood serum. Indeed, we have recently found that better *in vitro* multiplication and growth result during the first 24 hours by the perfusion technique if some serum is present in the nutrient medium. This fact emphasizes one of the basic reasons for our employment of two types of cultivation techniques. We find that the perfusion technique is particularly useful for the study of the nutrient requirements of the parasites, because any low molecular weight material essential for growth and not present in the nutrient fluid will be dialyzed away from the parasite and its deficiency rapidly made apparent. In the rocker-dilution technique, it is obvious that such deficiencies in the nutrient media will not be so readily observable. However, the rocker-dilution technique, because of its simplicity and lack of a Cellophane membrane, has proved very useful in testing the action of antimalarial drugs, immune serum, etc., on the growth of the malaria parasite *in vitro*.

Chemical and metabolic studies have also been made on parasites grown *in vivo* and *in vitro*. Increases have been observed in the content of fatty acids, flavine adenine dinucleotide, total phosphorus, 15-minute hydrolyzable phosphorus, phospholipid phosphorus and nucleic acid phosphorus (by difference) in the red blood cells as their parasite count increases either *in vitro* or *in vivo*. Similar studies on glucose and oxygen consumption and lactate production have also been carried out. We have encountered a striking difference between *in vitro* and *in vivo* grown parasites only in their oxygen consumption. Multiplication of parasites *in vitro* has not been attended by the same increase in oxygen consumption that is observed during multiplication *in vivo*. We have as yet no explanation for this phenomenon, but it may be a reflection of some deficiency or toxic agent in our media or an indication that, if the parasite is well supplied with nutrients, it can exist largely on energy derived from anaerobic processes. In support of the latter explanation is our finding that a gas phase low in O_2 (0.37 per cent. O_2: 5 per cent. CO_2: 94.63 per cent. N_2) permits at least as good growth and multiplication *in vitro* as in 95 per cent. air: 5 per cent. CO_2. Definitely detrimental to *in vitro* growth is a gas phase high in oxygen content (95 per cent. O_2: 5 per cent. CO_2).

The composition of the nutrient medium employed and given in Table 1 was arrived at by *a priori* reasoning. We can not say at present how many of the components of this medium are essential for growth of the parasite. Omission of the proteose peptone is, however, definitely detrimental to growth and multiplication. Recent experiments indicate that para-amino benzoic acid is probably the chief essential growth component furnished by the proteose peptone, a fact which may help to explain the observation of Coggeshall[5] that sulfanilamide will eradicate *P. knowlesi* infections in monkeys.

The techniques described here are also now being applied with the assistance of Dr. J. W. Ferrebee to the *in vitro* cultivation of human malaria parasites. Results to date have been encouraging and will be reported at a later time.

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THE ANTIBODY RESPONSE OF SWINE TO VACCINATION WITH INACTIVATED SWINE INFLUENZA VIRUS[1]

THE two human types of influenza virus, A and B, and the swine type of the agent have been obtained[2, 3, 4] in preparations of high purity by ultracentrifugation of the chorio-allantoic fluid of virus-infected chick embryos. A high degree of concentration and partial purification of the agents can be effected by sedimentation in the Sharples centrifuge,[5, 6] and procedures have been devised for practical large-scale production of virus for preparation of vaccines.[6, 7]

[5] L. T. Coggeshall, *Jour. Exp. Med.*, 71: 13, 1940.

[1] This work was supported through the Commission on Influenza and the Commission on Epidemiological Survey, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army. The work was aided also in part by a grant to Duke University from Lederle Laboratories, Inc., Pearl River, New York.

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[3] D. G. Sharp, A. R. Taylor, I. W. McLean, Jr., (Member, Commission on Epidemiological Survey, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army), D. Beard, J. W. Beard, A. E. Feller and J. H. Dingle, *Jour. Immunol.*, 48: 129, 1944.

[4] A. R. Taylor, D. G. Sharp, I. W. McLean, Jr., D. Beard, J. W. Beard, J. H. Dingle and A. E. Feller, *Jour. Immunol.*, 48: 361, 1944.

[5] W. M. Stanley, *Jour. Exp. Med.*, 79: 255, 1944.

[6] A. R. Taylor, D. G. Sharp, I. W. McLean, Jr., D. Beard and J. W. Beard, *Jour. Immunol.*, in press.

With access to purified, concentrated influenza virus, uantitative studies have been undertaken on the anti-ody-inducing capacity of influenza vaccines using wine as the test animal and the swine influenza virus the antigen. Swine are a natural host[8] for the wine influenza virus, which is closely similar morpho-gically[9] and physio-chemically[10,11] to the human ypes of influenza virus. In contrast with conditions man,[12] swine can be procured without previous xperience with the influenza virus, and experiments ith these animals under controlled conditions should ive a good estimate of the effects of the vaccine self.

The first study was made to determine the relation f virus mass (as vaccine) to host antibody response easured by the capacity of the serum taken at weekly ntervals to inhibit the hemagglutinin reaction.[13] wo types of vaccines,[14] namely, formalin-inactivated nd ultraviolet light-inactivated virus, were given sub-utaneously in 1 ml volumes in doses of 0.125, 0.25, .5, 1.0 and 2.0 mg of virus per ml. Each dose was iven to 12 pigs. It was found that within the range f dosage used, 0.1 to 5.0 mg per 100 lb of animal eight, a 10-fold increase in dose of the formalin accine produced only a 2.5-fold increase in titer, nd a similar increase in the dose of ultraviolet light accine resulted in a 1.6-fold increase in titer. The iter reached a peak within 7 to 9 days and then de-lined within 3 weeks to a level about three 2-fold ilution intervals below the maximum average titer ttained. Doses greater than 0.5 mg appeared to ause general reactions in the animals manifested hiefly by loss of weight.

These results showed clearly that though antibody iter was related to dose, the response was by no eans directly proportional to dose, and further the esponse was short-lived with any dose given. For his reason a second vaccination was made with the ame doses about 6½ weeks after first vaccination. The results were qualitatively similar to those of the first vaccination, and a 10-fold increase in dose of either vaccine was associated with a 1.6-fold increase in titer. In marked contrast with the results of a single vaccination, however, much higher titers were obtained, and the decline in titer subsequent to second vaccination was much slower. Higher and more last-ing titers were obtained with 0.25 mg of vaccine divided into two 0.125 mg doses, for example, than by an amount almost ten times as great given in a single injection. No significant difference was found between the effects of the vaccine prepared by the two methods of inactivation.

Further investigation was made of repeated vaccination particularly with respect to the optimum interval between the injections. For this, 64 pigs were given 0.5 mg of formalized virus, and at intervals of a week successive groups of 16 animals were revaccinated. The results showed that both the titer attained and the amount of antibody produced were greater the longer the interval between vaccinations. Furthermore, the greater the interval, the greater were the amounts of circulating antibody retained through the 6 weeks follow-up period. Repetition of vaccination after only 1 week produced little increase in titer over that following first vaccination. In fact, 7 of the 16 pigs revaccinated at the one week interval showed no increase, or an actual decrease in titer.

Experiments were made to test the possibility that higher titers might be obtained and maintained by vaccinating with vaccine adsorbed on alum. The effects of a single injection of 0.5 mg of formalized virus adsorbed on different amounts of alum, from 0.3 to 1.2 per cent. were similar in magnitude to those of a single injection of vaccine without alum. A striking difference was the delay of peak response until the second week post-vaccination; following this, antibody was lost at nearly the usual rate. The height of the peak response increased as the concentration of alum decreased, but with the highest concentration of alum tested a somewhat higher and more uniform titer was maintained throughout the 7 weeks follow-up period. All the animals showed areas of local induration at the site of inoculation that gradually subsided, but could still be detected in nearly all instances at the end of seven weeks.

Vaccinations repeated at two and four weeks with 0.5 mg of virus adsorbed on 1.2 per cent. alum gave results similar in magnitude to those obtained with vaccine containing no alum. Here, also, two vaccinations, four weeks apart, gave the highest titers, and the decline in titer was not much slower than that in animals receiving vaccine without alum. It appears doubtful that the use of alum influenced significantly either the antigenic capacity of formalinized swine influenza virus or the maintenance of antibody titer.

The findings reveal an efficiency of repeated small doses of vaccine in swine far greater and longer-lived than that of a single large dose, and a considerable dependence of the degree of effect on the length of the interval between vaccinations. Formalin and ultraviolet vaccines behaved alike, and adsorption on

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alum did not greately enhance the titer or prolong the antibody level.

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ROOTING GREENWOOD CUTTINGS WITHOUT SUNLIGHT UNDER FLUORESCENT LAMPS

OVER a century ago, improvement in heating devices led to the change from opaque structures with some glass-covered openings to the greenhouse of the present. The improved illumination thereby obtained seemed to compensate for the increased fuel requirement and more frequent watering and more constant care this change entailed. For many years, though fuel and labor costs have increased markedly and new types of illumination have been developed, there has been no essential change in the type of house used for growing plants, and propagation of plants has been carried on in the same houses, or slight modifications of them, as are used for growing. High fuel costs in winter and excessive insolation in summer have been accepted unquestionably.

Recent studies made at the U. S. Plant Introduction Garden of the Division of Plant Exploration and Introduction, U. S. Department of Agriculture, Glenn Dale, Maryland, indicate the desirability of investigation of the possibility of using opaque structures for the rooting of cuttings. Such structures if partly or wholly underground could be heated economically in winter and also could avoid the high temperatures which are often a source of difficulty in the summer.

Some experiments conducted during the summer of 1942 indicated that more rapid rooting of cuttings of *Weigela floribunda* (Sieb. and Zucc.) C. A. Mey, *Ligustrum ovalifolium* Hassk. and *Chrysanthemum morifolium* Ram. could be obtained in a basement darkroom under continuous illumination from a 100 watt mazda lamp than in conventional propagation equipment in a greenhouse. In a continuation of these experiments using more efficient lighting equipment, particularly good results were obtained with small detached propagation cases of opaque material, each fitted with a single 30 watt fluorescent lamp. These cases were approximately six feet in length, two feet wide and three feet high, and the lamp was placed 10 inches above the rooting medium, which in this instance was a coarse grade of vermiculite. Automatic control of the bottom heat in the rooting medium was provided by means of a lead-sheathed soil-heating cable controlled by a thermostat. The small amount of heat given off by the lamp was absorbed largely by the air space above it and did not raise the temperature of the air around the cuttings. In other experimental frames using lamps of higher wattage, the cuttings were protected from heat by means of a glass partition between the cuttings and the lamp. Both the white and the daylight quality lamps have been used successfully. The operating cost was low because of the high efficiency of the fluorescent lamps.

Uniform relative humidity (approx. 80 per cent.) was maintained easily within the cases because of the constant light and temperature (75° F.). In the conventional greenhouse, on the other hand, the constant fluctuations cause serious difficulty in maintaining a uniform humidity.

The results obtained in rooting cuttings in these frames have been exceptionally good, and have surpassed those in similar propagating frames within an ordinary greenhouse, when the rooting medium and the temperatures have been the same in both locations. The cuttings tried include those of various species of *Citrus, Cinchona, Severinia, Hibiscus, Bougainvillea* and unrooted divisions of *Cymbopogon citratus* DC.

In comparing rooting under 16-hour daily photoperiods with that obtained under continuous illumination, the responses were often superior in one or the other day-length according to the individual species. The difference was negligible with cuttings of some plants. These responses can be changed by treatments with growth substances in some manner not explicable at present. For instance, cuttings of the Chinotto orange, *Citrus Aurantium* Linn. var. *myrtifolia* Ker-Gawl., following a treatment by dipping the bases in a 50 per cent. solution of ethyl alcohol containing five mg of potassium indole butyrate per ml rooted heavily under a 16-hour photoperiod in only two weeks, but rooting was slight and greatly delayed under continuous illumination. Moderate and approximately equal rooting of untreated cuttings was obtained in both day-lengths. On the other hand, the identical treatment of growth substance on cuttings of rough lemon, *Citrus Limon* Linn., produced a heavy rooting response under continuous illumination, but rooting was inhibited under the 16-hour photoperiod. As with the Chinotto orange, the rooting of the untreated cuttings was practically equal under both periods of illumination.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR QUANTITATING THE PRECIPITIN TEST[1]

In March, 1944, the authors described a method for quantitating the precipitin test.[2] Its extended use has led to several important improvements, and it has been found applicable to the Kahn test and to other precipitin reactions. The accuracy of the method is enhanced by the fact that the process is carried out in a Meloche[3] tube on which optical measurements are made.

The Meloche tube[4] is so constructed that the bowl, 6.5 cm, quickly tapers into a capillary stem of 5 cm length with a bore of approximately 0.4 mm. It is necessary that the diameter of the capillary of each tube be calibrated separately. Mercury is used in this laboratory for this purpose.

Through the lower end of the capillary, a column of mercury is drawn up about 1 cm, and the tip of the tube is then sealed with dental sticky wax. Antigen and specific immune serum in appropriate amounts are measured into the bowl, which is then covered with a paper cap. The tube is placed in a shaking machine for two minutes, then in a water bath at 37° C for one hour and then at icebox temperature overnight. The bowl is shaken gently and the tube placed in a centrifuge cup, which is cushioned with heavy rubber tubing to prevent tilting. Centrifugation is carried out at 2,500 r.p.m. for 15 minutes during which all the precipitate packs to a maximum degree in the capillary tube adjacent to the mercury seal. The length of this column of precipitate is then measured in a simple optical apparatus.

The optical system consists of a brass tube 20 cm in length and 2.5 cm in diameter. The specifications of the eye cap is +7 diopters and of the adjacent eyepiece +100 diopters. A lower lens, which is at a distance of 11 cm, has a power of −7 diopters. The magnification of the lens system is, therefore, ×10. A micrometer scale which is calibrated against a known scale is attached to the upper eyepiece. In the apparatus in use, 1.0 mm at the point of focus is equivalent to 25 micrometer scale units.

The following factors enter into the calculation of the apparent volume of precipitate in terms of cubic millimeters:

The length of the column of precipitate (in micrometer units) ..... A

½ diameter of capillary, squared × 3.1416 (πr^2) ..... B

The magnification of the micrometer scale C

$$\frac{A \times B}{C}$$ ——— volume of precipitate in cu mm.

The accuracy of the method was found to be less than 0.01 cu mm.[5]

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THE INACTIVATION OF "MARFANIL" [SULFAMYLON] BY TISSUE SLICES IN VITRO[1]

In a recent article Beyer and Govier[2] concluded that "marfanil" (p-amino-methyl benzene sulfonamide) is oxidatively deaminated by the amine oxidase. They used guinea pig liver suspension. We have studied the inactivation of "marfanil" with the tissue slice technique in rat and guinea pig liver and kidney. Three hundred mg of tissue slices were incubated in 4.0 cc of Krebs' bicarbonate solution in an atmosphere of 95 per cent. oxygen and 5 per cent. carbon dioxide. The drug was estimated in the Van Slyke apparatus, at the end of the incubation period, after the addition of 1.0 cc of 20 per cent. trichloroacetic acid and the removal by centrifuging of the precipitated protein. Somewhat more than half of the nitrogen of the drug is given off in the Van Slyke apparatus and the amount varies in the presence of different tissue extracts and increases slightly after the drug has been autoclaved. Consequently, four estimations were necessary to determine the amount of drug inactivated. These were estimations (a) on extracts from tissue slices incubated alone; (b) on extracts from tissues to which 1.0 mg of the drug was added immediately before the addition of the trichloroacetic acid; (c) on extracts from tissue with which 1.0 mg of the drug had been incubated; (d) the same as (c) except that 1.0 mg more of the drug was added immediately before the trichloroacetic acid. When the control value (a) is subtracted, the sum of the values obtained from (b) and (c) should equal (d). Table 1 gives a typical experiment with the four tissues studied. Rat liver does not inactivate the drug, whereas the other three tissues do. Rat liver, however, contains more amine oxidase than rat kidney. Recent work[3, 4, 5] has indicated that there

[5] This work was supported by a grant from the John and Mary R. Markle Foundation.

[1] The marfanil or sulfamylon was kindly supplied by Dr. F. B. Rice of the Winthrop Chemical Co.

[2] K. H. Beyer and W. M. Govier, Science, 101: 150, 1945.

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[1] From the Department of Medicine, Washington University School of Medicine, and The Oscar Johnson Institute for Research, St. Louis, Mo.

[2] M. C. Johnson, H. L. Alexander, R. Robinson and J. H. Alexander, *Jour. Allergy*, 15: 83, 1944.

[3] V. W. Meloche and J. Y. Yen (University of Wisconsin), personal communication.

[4] Meloche tubes may be purchased commercially (Corning Pyrex No. 8085) but, because of priorities and delayed deliveries, may be blown to specifications.

TABLE 1

THE INACTIVATION OF 1.0 MG. "MARFANIL" BY 300 MG. OF TISSUE SLICES AFTER 3 HOURS INCUBATION AT 37°

Tissue		Mg. N_2	Difference	Per cent. Inactivation
Rat kidney	a	0.267		
	b	0.340	0.073	
	c	0.293	0.026	65
	d	0.365	0.098	
Rat liver	a	0.245		
	b	0.322	0.077	
	c	0.317	0.072	6
	d	0.402	0.157	
Guinea pig kidney	a	0.205		
	b	0.297	0.092	
	c	0.262	0.057	38
	d	0.345	0.140	
Guinea pig liver	a	0.178		
	b	0.255	0.077	
	c	0.225	0.047	39
	d	0.305	0.127	

(a) is the value obtained from the tissue alone; (b) 1.0 mg added at the end of the incubation period (c) 1.0 mg added at the beginning of the incubation period; (d) 1.0 mg added at the beginning and then 1.0 mg at the end of the incubation period.

are several enzymes in the body which inactivate amines and possibly one of these or a still unidentified enzyme is responsible for the inactivation of the drug. Autoclaving the drug and tissue mixture of 20 pounds pressure for 30 minutes in strong acid after incubation causes no increase in the amino nitrogen, indicating that conjugation does not occur. The reaction does not proceed under anaerobic conditions.

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FILTRATION ADAPTER[1]

THE usual technique in performing a bacteriological filtration requires that the material be passed through a bacteriological filter, such as the Berkefeld, Chamberland, Seitz or fritted glass type, which in turn is mounted on a filtrate receptacle. If the sterile filtrate, thus prepared, is to be stored for any time, it must then be transferred, aseptically, to a storage bottle. This last operation increases the danger of contamination and can be eliminated if the sterilizing filtration can be made directly into the final receptacle.

An excellent way to perform such filtrations has been suggested by Morton,[2] who combines in one piece a fritted glass filter and a suction arm. This apparatus may be united by means of a ground-glass joint to a filtrate receptacle which is an ordinary Erlenmeyer flask with a tapered male ground-glass joint.

While the filtrate receptacle in this apparatus may be used as a storage container for the sterile filtrate, it does not lend itself to this use too readily. Firstly, the taper of the ground-glass joint will not allow use of a cotton plug or a rubber vaccine cap. Secondly, one would be required to stock a great num and variety of sizes of ground-glass jointed flask meet the normal requirements of a laborat

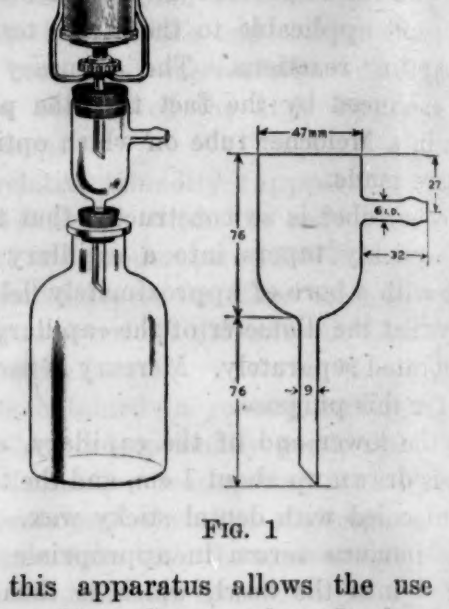

FIG. 1

Finally, this apparatus allows the use only o fritted glass filter, the ceramic and asbestos types being applicable.

The simple adapter illustrated above is desig to overcome these disadvantages. It will allow the of any type of standard filter which may then be u with any type of storage receptacle, provided t this receptacle can withstand vacuum.

Thick-walled pyrex bottles suited for sterilizat which are commonly used for storage of sterile liqu should serve as well for this purpose as do the us filter flasks with side arms.

The use of this adapter makes unnecessary the tempt to mount the filter in a two-holed rubber st per which, with small-necked bottles, is impossi and, in any case, awkward and unwieldy. The ad ter is equally useful for chemical filtrations us Buchner or conical funnels.

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[1] The adapter illustrated in this article was blown by the Emil Greiner Company of New York and is available from them.

[2] H. E. Morton, *Jour. Bact.*, 47: 379, 1944.

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